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Remote Kidney and Liver Injury After Transplantation of Lung Allografts in an Allogeneic Mouse Model

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ABSTRACT

Background. Remote organ dysfunction is common after lung transplantation and might negatively affect the outcome. The local anesthetic ropivacaine was previously demonstrated to attenuate acute rejection after allogeneic lung transplantation in mice. We hypothesized that lung transplantation might result in detectable molecular signs of injury in kidneys and liver and that ropivacaine might attenuate this damage.

Methods. Organs from C57BL/6 mice undergoing allogeneic orthotopic single-lung transplantation were procured at postoperative day 5 and analyzed using Western blot and real-time quantitative polymerase chain reaction probing for Src protein tyrosine kinase, STAT3, and bax/bcl-2. During cold ischemia, the allograft had either been flushed with normal saline only or in combination with ropivacaine (1 μM). A nontransplanted group of animals served as the baseline controls.

Results. The allogeneic stimulus induced by transplantation led to an increase in Src-phosphorylation and STAT3-expression in the kidneys and livers of lung-transplanted mice compared to nontransplanted animals. Bax/bcl-2 as a marker of cellular apoptosis was not affected by the transplantation. In contrast to the findings in the transplanted lungs, the addition of ropivacaine did not have an effect on the examined markers of inflammation in the remote organs.

Conclusions. The observed increase in the inflammatory signaling provides first insight into a possible mechanism, by which remote organ dysfunction after lung transplantation might occur.

LUNG transplantation is a well-established surgical treatment for various end-stage lung diseases [1]. However, despite substantial progress regarding treatment options, median survival after lung transplantation remains low in comparison to transplantation of other solid organs [1]. The most common complications after lung transplantation are organ rejection, (bacterial) infections, and adverse effects of immunosuppressive drugs [1]. Additionally, organ dysfunctions, such as acute liver failure (ALF) and acute kidney injury (AKI), might occur during the postoperative course after lung transplantation with limited treatment options available, thus decreasing overall survival [2,3]. Depending on the definition used, ALF occurs in 7% to 28% of patients who undergo lung transplantation [3]. AKI, defined as a sudden and potentially reversible loss of renal function, is a common complication after lung transplantation [4,5]. With an incidence of approximately 65%, AKI is 3 to 4 times more frequent in lung transplant recipients than after any other comparable major thoracic

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surgery [2]. However, the exact mechanisms leading to either ALF or AKI after lung transplantation remain elusive, although a potential role of both prolonged hypoxemia in combination with ischemia-reperfusion injury and oxidative stress has been proposed.

Recent evidence suggests that systemic inflammation and consecutive endothelial damage might contribute to AKI and ALF in transplant recipients [2]. There are several pathways, by which the inflammatory response might be triggered. In human transplantation, ischemia-reperfusion (I/R) injury during transplant surgery might result in a dysfunctional endothelium with increased permeability and subsequent organ damage [6,7]. The I/R not only affects the organ undergoing the initial ischemia but has also impact on remote organ systems which may lead to damage, a phenomenon coined as remote organ injury (ROI) [8]. However, in allogeneic, experimental (mouse) models of transplantation − such as the one presented in the current study [9] − the allogeneic stimulus caused by the transplantation of the organ from another strain will also lead to a strong increase in inflammatory mediators and cells and induce migration into the graft, ultimately leading to activation of macrophages and T lymphocytes [7,10,11]. The secretion of pro-inflammatory cytokines − such as tumor necrosis factor α (TNF-α) − by these cells further aggravates endothelial permeability through the activation of Src protein tyrosine kinase (Src) in endothelial cells [12]. Other pro-inflammatory cytokines such as interleukin (IL)-6 are also known to activate the signal transducer and activator of transcription (STAT)3, which is another key regulator of the organism's response to inflammatory stimuli caused by the allogeneic transplantation [11,13].

Amide-linked local anesthetics (LAs), such as ropivacaine, exert strong anti-inflammatory properties and have been shown to be able to attenuate endothelial dysfunction [12,14], while maintaining immune function [15]. In a mouse model of experimental, orthotopic (single) lung transplantation, pretreatment of the grafts with ropivacaine lead to reduction of TNF-α in plasma 3 hours after transplantation, a reduction of acute rejection and the inhibition of phosphorylation of the Src target protein caveolin-1 within the transplanted lung 5 days after surgery [6]. In the present study, we evaluated the kidneys and livers procured 5 days after transplantation from the same lung-transplanted mice, which had been assessed for pulmonary graft function and rejection in the primary investigation [6]. In order to generate new research questions in the field of solid organ transplantation, two specific aims were set for this particular investigation: (1) to assess whether there might be a detectable molecular inflammatory response in the kidneys and livers of the transplanted animals presumably caused by the allogeneic stimulus, and (2) to evaluate whether this response might possibly be attenuated by the LA ropivacaine.

MATERIALS AND METHODS

Declarations

This study was approved by the respective local animal care committee (protocols no. ZH103/2016 for transplant experiments and T10/18 for baseline animal data). All experiments were conducted in accordance with the ARRIVE guidelines. Animals received adequate care according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Experimental Procedure and Procurement of Organs

Organs (livers and kidneys) used for this study were procured from 10 to 14-week-old C57BL/6 male mice who earlier [6,9] received an orthotopic, single left lung transplantation from BALB/c mice donors (both acquired from Charles River Laboratories, Sulzfeld, Germany). The results of the assessment of the lungs of these animals has already been reported [6]. In order to be consistent and to be able to compare results among animals and to avoid potential sex-dependent differences in the respective end points, we have chosen to conduct a single-sex study, in this particular case, with male animals. However, the choice of the sex was arbitrary and the study could also have been conducted using female animals only.

Kidneys and livers were procured on postoperative day 5 and were immediately immersed in liquid nitrogen and stored at −80 °C until further analysis. There were three groups of animals in total (two within the transplanted population and one untreated baseline):

1. **Control group:** Here, donor lungs were flushed with a preservation solution containing normal saline (0.9%) prior to surgical removal.
2. **Ropivacaine group:** Ropivacaine at a final concentration of 1 μM was added to the preservation solution (0.9% normal saline) in this particular group. In both groups, the fluid remained in the donor organs for 1 hour, during which the transplants were stored at 4 °C (cold ischemia time). During the transplantation, the preservation solution containing ropivacaine or normal saline only was flushed into the recipient's bloodstream.
3. **Baseline group:** For the current study, an additional baseline group of healthy, untreated, 10 to 15-week-old C57BL/6 male mice was established. Those mice were euthanized by cervical dislocation, then, their organs were recovered and immediately snap-frozen in liquid nitrogen and stored at −80 °C.

Western Blot Analysis

For Western blot analysis, pieces of the organs were lysed using a lysis-buffer (NaCl 140 mM, Triton X-100 1.5% v/v], Deoxycholic acid 0.5% [w/v], SDS 0.1% [w/v], NaF 1 mM, Na_3VO_4 1 mM, Tris/HCl [pH 7.4] 40 mM, all the chemicals were purchased from Carl Roth, Karlsruhe, Germany) including Roche protease inhibitor cocktail complete mini EDTA-free (Roche Diagnostics, Mannheim, Germany). Protein concentrations were measured using the Bradford method with bovine serum albumin (BSA) as standard (Sigma-Aldrich, St. Louis, MO, USA). SDS-PAGE was performed to separate proteins using polyacrylamide-gels (8%, 10%, and 12%, depending on the

protein of interest). Antibodies against phospho-Src (pY418, Cell Signaling, Danvers, MA, USA; #2101), total Src (Santa Cruz Biotechnology, Dallas, TX, USA; sc-8056), STAT3 (Cell Signaling #9139), and GAPDH (Abcam, Cambridge, UK; ab8245) were used as primary antibodies for the Western blot analysis. Horseradish peroxidase conjugated goat anti-rabbit or anti-mouse antibodies (both from Jackson Immunoresearch, Ely, UK) were used for detection, which was conducted using the SuperSignal West Pico PLUS Chemiluminescent Substrate kit (Thermo Fisher Scientific, Waltham, MA, USA) and the MicroChemi 4.2 imager (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel). Densitometry was carried out using ImageJ software (https://imagej.nih.gov/ij/).

Real Time Quantitative Polymerase Chain Reaction

For quantitative polymerase chain reaction (qPCR) analysis, total ribonucleic acid (RNA) was extracted using the E.Z.N.A. Total RNA Kit I (Omega Bio-tek, Norcross, GA, USA). The final concentration of RNA in the samples was measured using the NanoDrop One UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was performed on equal amounts of RNA (500 ng and 1000 ng) with Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT, Promega Corporation, Madison, WI, USA) using oligo-dT (20 nt) primers. The resulting cDNA was examined for the level of B-cell lymphoma 2 (bcl2), Bcl-2-associated X protein (bax) expression using a SYBR Green I Master Mix and a LightCycler 480 System (Roche Diagnostics, Mannheim, Germany). The obtained bcl2/bax ratio was used as a marker for cell apoptosis. Primers specific for the respective transcripts of bcl2 (sequence 5′ → 3′ forward ATGCCTTTGTGGAACTATATGGC, reverse GGTATGCACCCAGAGTGATGC), bax (sequence 5′ → 3′ forward TGAAGACAGGGGCCTTTTTG, reverse AATTCGCCGGAGACACTCG) were obtained from Metabion International AG (Planegg, Germany).

Statistical Analysis

Data were assessed for normal distribution using a Shapiro-Wilk test. All data were analyzed using a Kruskal-Wallis test with Dunn's correction for multiple comparisons and the results are reported as median with interquartile range (IQR) in the text or as boxplots (median with quartiles, whiskers representing minimum and maximum) in the figures. Statistical analysis was performed with GraphPad Prism for macOS (version 9.1.1; GraphPad Software, San Diego, CA, USA). A P value less than .05 was considered to be statistically significant.

RESULTS

Activation of Src Protein Tyrosine Kinase in Kidneys Five Days After Lung Transplantation

Western blot analysis of kidney homogenates showed that lung transplantation led to an increased Src expression in the kidneys of the transplanted animals compared to nontransplanted (baseline) animals on postoperative day 5. Median Src expression was 2.6-times higher in the control group compared to the baseline group ($P = .04$; Figs 1Ai and 1Aii). The addition of ropivacaine did not have an effect on Src expression compared to control animals (control group median 2.56 vs ropivacaine group median 2.48, $P > .99$; Fig 1Aii).

Lung transplantation also led to a significant increase (6.3-fold) of Src phosphorylation at tyrosine 418 (pY418 Src) in the kidneys of lung-transplanted mice compared to baseline on postoperative day 5 ($P = .002$). There was no significant effect on Src phosphorylation by the addition of ropivacaine ($P > .99$; all see Figs 1Ai and 1Aiii).

Activation of Src Protein Tyrosine Kinase in Livers Five Days After Lung Transplantation

As shown in Fig 1B, there was no statistically significant difference in median total Src expression in lysates from livers of the baseline, control, and ropivacaine groups (baseline group median 1.02 vs control group median 1.13, $P = .61$; and control group median vs ropivacaine group median 1.7, $P > .99$). However, median Src phosphorylation at tyrosine 418 (pY418) was significantly higher (15.9-fold) in the livers of lung-transplanted mice compared to baseline on postoperative day 5 ($P = .001$). The addition of ropivacaine to the preservation solution did not have an effect on Src phosphorylation when compared to the control group ($P > .99$; Figs 1Bi und 1Biii).

Expression of STAT3 in Kidneys and Livers Five Days After Lung Transplantation

The results of the Western blot and densitometry analysis regarding STAT3 expression are shown in Fig 2A. Median STAT3 expression in the kidneys increased by a factor of 1544 in the control group when compared to the non-transplanted baseline group ($P < .001$). Ropivacaine pretreatment of the allograft did not have an effect on median STAT3 expression in the kidneys of the lung-transplanted animals ($P > .99$; Figs 2Ai and 2ii).

In the livers, lung transplantation led to an increased median STAT3 expression (17.3-times increase) compared to the non-transplanted baseline group ($P < .0001$). Mean STAT3 expression in the livers of the lung-transplanted animals was not affected by ropivacaine pretreatment of the allograft ($P > .99$; Fig 2B).

Bax/Bcl2 Ratio in Kidneys and Livers Five Days After Lung Transplantation

In both the kidneys and livers of the transplanted animals, the median Bax/Bcl2 ratio on postoperative day 5 did not significantly vary as compared to the non-transplanted baseline group (kidney = baseline 29.4 and control 23.8, $P > .99$; and liver = baseline 135 and control 105.5, $P = .9$). Furthermore, in both organs, ropivacaine pretreatment of the allograft did not have a significant effect on the mean Bax/Bcl2 ratio (kidney = control 23.8 and ropivacaine = 18.6, $P = .99$; and liver = control 105.5 and ropivacaine = 165, $P = .5$; Fig 3).

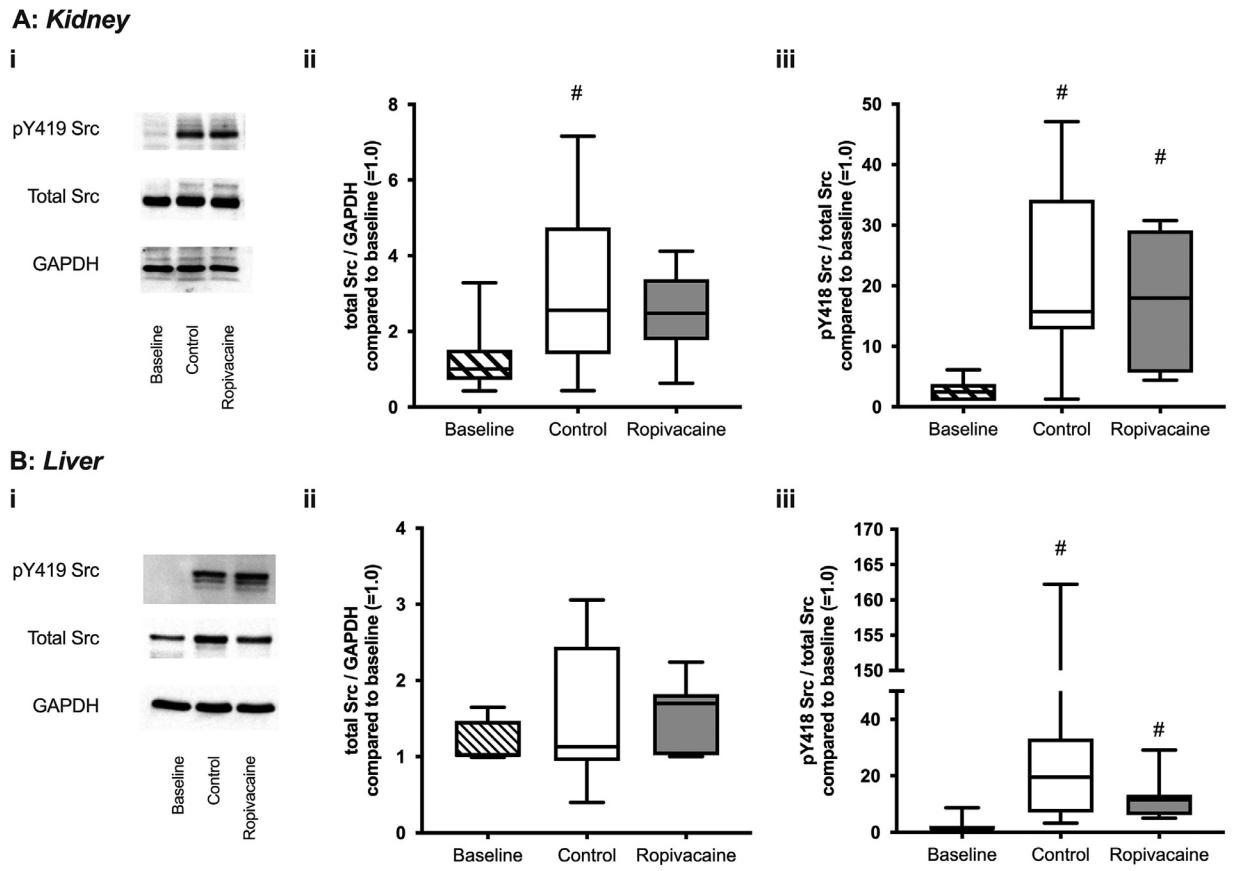

Fig 1. **(Ai)** Representative Western blots of phosphorylated Src protein tyrosine kinase (pY419 Src, row 1), total Src (row 2), and GAPDH (row 3) in kidneys of nontransplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). **(Aii)** Quantification of densitometry analysis of Western blots. Ratios of total Src over GAPDH, normalized to values in the baseline group (=1.0), are shown. Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline (striped white boxplot) and control groups (white boxplot), and seven for the ropivacaine (grey boxplot) group. # $P < .05$ vs baseline. **(Aiii)** Quantification of densitometry analysis of Western blots. Ratios of pY418 Src over total Src, normalized to values in the baseline group (=1.0), are shown. **(Bi)** Representative Western blots of phosphorylated Src protein tyrosine kinase (pY418 Src, row 1), total Src (row 2), and GADPH (row 3) in livers of nontransplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). **(Bii)** Quantification of densitometry analysis of Western blots. Ratios of total Src over GAPDH, normalized to values in the baseline group (=1.0), are shown. Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline (striped white boxplot) and control groups (white boxplot), and seven for the ropivacaine (grey boxplot) group. **(Biii)** Quantification of densitometry analysis of Western blots. Ratios of pY418 Src over total Src, normalized to values in the baseline group (=1.0), are shown. Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine sampels for the baseline (striped white bar) and control (white bar) groups, and seven for the ropivacaine group (grey bar). #$P < .05$ compared to baseline.

DISCUSSION

The main finding of this experimental study analyzing livers and kidneys procured from mice 5 days after allogeneic lung transplantation was a significant increase in the expression of key regulators of inflammation, such as Src kinase and STAT3. However, in contrast to the transplanted lungs [6], the addition of ropivacaine did not change the expression of the assessed proteins in both remote organs. The observed increase in inflammatory signaling does not necessarily mean that the organs were affected in their function or that a severe structural damage has occurred, which is of course also a major limitation of this type of analysis.

It is already known that inflammatory injury occurs during and after lung transplantation, which might not only lead to consecutive systemic inflammation, but also to remote organ dysfunction: inflammatory events might have an impact on remote organ systems, which might in turn lead to their damage [8]. The ROI is substantially caused by the same mechanisms, which induce the injury in the primarily affected organ. Here,

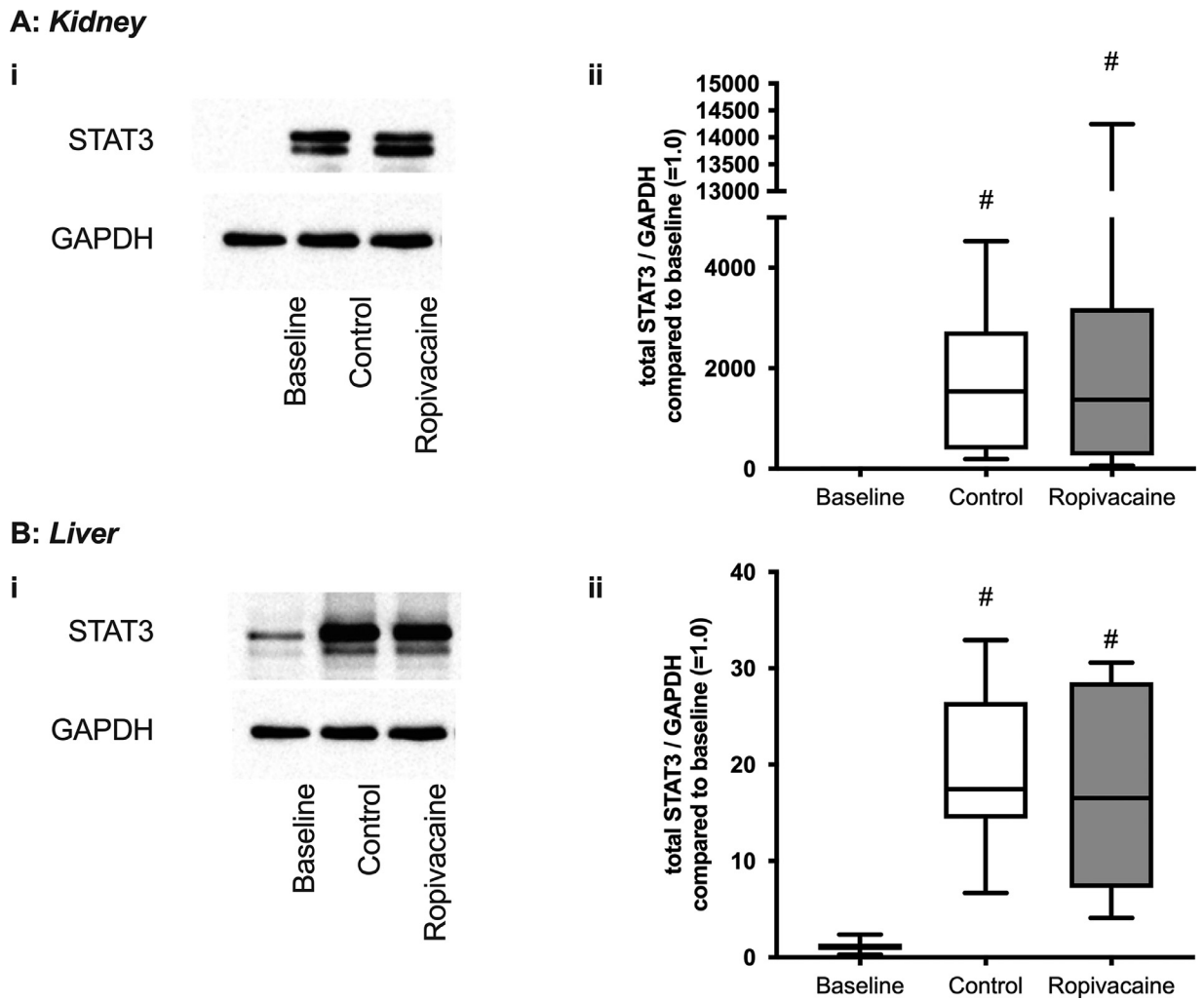

Fig 2. (**Ai**) Representative Western blots of STAT3 (row 1) and GAPDH (row 2) in kidneys of nontransplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). **(Aii)** Quantification of densitometry analysis of Western blots. The ratio of STAT3 over GAPDH, normalized to values in the baseline group (=1.0), are shown. Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline (striped white bar) and control (white bar) groups, and seven for the ropivacaine group (grey bar). #$P < .05$ compared to baseline. **(Bi)** Representative Western blots of STAT3 (row 1) and GAPDH (row 2) in the kidneys of nontransplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). **(Bii)** Quantification of densitometry analysis of Western blots. The ratio of STAT3 over GAPDH, normalized to values in the baseline group (=1.0), are shown. Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline (striped white bar) and control (white bar) groups, and seven for the ropivacaine group (grey bar). #$P < .05$ compared to baseline.

inflammatory cells, cytokines, and reactive oxygen species (ROS) are the key factors [8]. The allogeneic stimulus in the experimental model not only leads to an increase of pro-inflammatory cytokines, such as TNF-α and IL-6, but also to the activation of T lymphocytes and macrophages [11], which then induce a severe systemic inflammatory response [16].

Furthermore, TNF-α activates Src [12], a phenomenon which plays a key role in the development of endothelial dysfunction by increasing endothelial permeability, subsequently resulting in tissue and organ injury [12]. Consistently, an increase in Src activity was found in the respective affected organs in various models of experimental inflammation, for example, in a rat model of an experimental lung [17], but is also important during the development of AKI [18,19]. In the current study, increased Src activation was also observed in the kidneys and livers 5 days after transplantation, although these remote organs did not undergo transplantation. Here, lung transplantation led to upregulation of Src phosphorylation – an indicator for

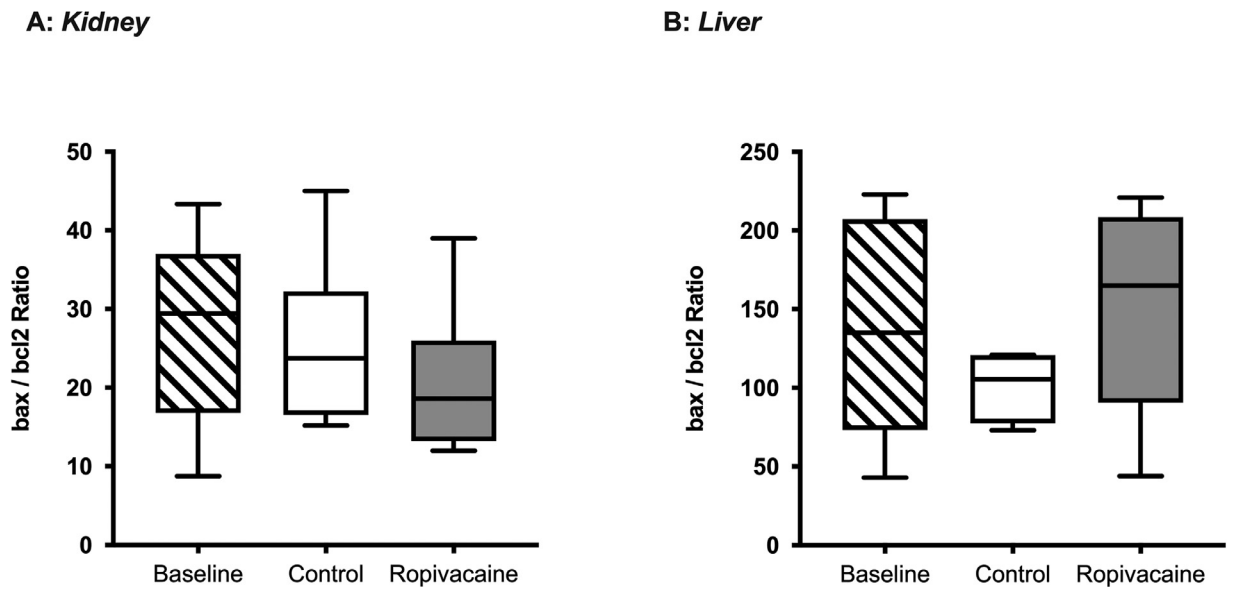

Fig 3. (A) Bax/bcl2 ratio in kidneys of non-transplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline group (striped white bar), eight for the control group (white bar), and seven for the ropivacaine group (grey bar). **(B)** Bax/bcl2 ratio in livers of non-transplanted animals (baseline) and lung-transplanted mice at postoperative day 5, whose allograft was flushed with normal saline only (control) or with normal saline plus ropivacaine at a final concentration of 1 μM (ropivacaine). Data are presented as boxplots (median with quartiles, whiskers representing minimum and maximum). There were nine samples for the baseline group (striped white bar), four for the control group (white bar), and five for the ropivacaine group (grey bar).

increased Src activity – in both the kidneys and livers. In addition, a significant upregulation of Src expression in the kidneys could be demonstrated, whereas, in the liver, a similar trend toward higher Src expression without reaching statistical significance was found. These results might be at least in part explicable by the effects of the inflammatory response as induced by the allogeneic stimulus and might therefore be interpreted as signs of ROI, although the exact mechanism remains elusive at this point. In the previously published study evaluating the lungs of the transplanted animals, mean serum TNF-α levels were elevated 3 hours after transplantation [6]. Hence, the increased activation and expression of Src in the kidneys and livers after lung transplantation might have been mediated – at least in part – by TNF-α.

Pretreatment of the lung allograft with the amide-LA ropivacaine proved to be beneficial by attenuating acute rejection and decreasing TNF-α serum levels [6]. Ropivacaine exerts strong anti-inflammatory properties and has already been shown to attenuate endothelial dysfunction in vitro and in vivo, mainly through inhibiting Src activation and its downstream signaling events [12,14]. Interestingly, it is also known that ropivacaine is able to inhibit the priming of neutrophils [20], a phenomenon involved in the pathogenesis of ROI [8]. In the current study, Src activation and expression in the kidneys and livers of both lung-transplanted groups was not significantly attenuated by the addition of ropivacaine to the preservation solution. In the lung transplantation model used, the remaining ropivacaine in the allograft was flushed into the bloodstream of the recipient mice after reperfusion and therefore diluted immediately. Hence, the systemic concentration, which was able to reach the kidney or liver could be expected to be much lower than in the lungs and – in accordance with the observed results – most probably too low to exert anti-inflammatory effects in those remote organs. The lack of difference in Src activation and expression between the two groups also occurred although lower serum TNF-α levels in the ropivacaine group were measured [6]. A possible reason for this observation could be that TNF-α is not the only cytokine upregulated after transplantation and that other, that is, TNF-α-independent, mechanisms involved in Src activation, for example, by ROS or other inflammatory cytokines like IL-6 might have contributed to a similar degree of Src activation and expression [21], which then could not be attenuated by ropivacaine. This is also in accordance with the previously published finding that ropivacaine in experimental lung transplantation of the same animals (from which the assessed kidneys and livers originated) decreased the serum level of TNF-α and increased the release of the anti-inflammatory cytokine IL-10, but was not able to alter the transplantation-associated increase in IL-6 [6].

Increased STAT3 expression was observed in both the kidney and liver samples from transplanted animals as compared to the non-transplanted baseline group. STAT3 is a member of the STAT family of proteins, which are well-known transducers of signals from growth factors and cytokines to the cell nucleus [13]. Activation of STAT3 has been demonstrated in various inflammatory injury models, including tissues undergoing allogeneic transplantation [11]. The current study is again

able to extend these findings by examining remote organs after a transplantation-related allogeneic immune stimulus to the immune system, which is known to promote the release of pro-inflammatory cytokines, such as IL-6 from monocytes and macrophages [22,23]. IL-6 not only activates STAT3 by phosphorylation [13], but also induces the transcription of the stat3 gene, thus increasing the levels of STAT3 [24,25]. As reported previously, serum levels of IL-6 were elevated in both lung-transplanted groups of the animals analyzed in this study [6,26]. It is therefore likely that IL-6 might have caused an increased STAT3 expression in the kidneys and livers as observed. Again, this clearly confirms our previous findings that allogeneic transplantation induces the same pathways both locally and to remote organs, which are likely to cause their damage. In our study, higher IL-6 serum levels did not correlate with an increased STAT3 expression in kidneys and livers, which could again be explained by the assumption that lower IL-6 levels could already have caused the same extent of STAT3 expression and that further mechanisms of STAT3 upregulation were involved.

The degree of cellular injury was investigated by measuring expression levels of pro- and anti-apoptotic genes, like Bax and Bcl-2. The Bax to Bcl-2 ratio has been shown to positively correlate with cell susceptibility to apoptosis [27]. In the current study, significant differences between the groups by measuring the Bax/Bcl2 ratio could not be observed. It therefore remains unclear, whether the observed transplantation-related inflammatory response in the kidneys and livers might also have caused cellular damage by apoptosis contributing to a potential organ injury and, unfortunately, this aspect cannot be addressed in total by the current analysis.

Limitations

The experimental mouse model of orthotopic (single) lung transplantation used in this study has been very well described [9], but all observations should still be interpreted with caution as their transferability into human biology might be limited [28]. Additionally, no sham group has been included in the experimental setup. Thus, a differentiation between effects induced by the allogeneic stimulus rather than a surgical stimulus only, for example, by an incision of the skin, could be difficult. However, despite these limitations the model remains the gold standard for preclinical lung transplantation research and its use for a hypothesis-generating study like the current one might still be justified.

Another limitation might also be the fact that an assessment of cellular damage of remote organs secondary to the observed inflammation is not possible from the presented data. The current study is a secondary workup of liver and kidney samples from animals originating from the previously published study investigating the effects of ropivacaine on acute rejection in transplanted lungs [6] and the availability of animal sample material was strictly limited. Additionally, due to the humane end point defined in this study, all animals were euthanized already 5 days after the transplantation. This time point might be too early to expect a fully developed or clinically relevant kidney or liver failure, respectively [29].

CONCLUSIONS

The current study, which has been designed as exploratory, hypothesis-generating, experimental research, extends the understanding of systemic inflammation after allogeneic experimental lung transplantation. The data clearly show that lung transplantation results in an increased activation and expression of pro-inflammatory proteins in both the kidneys and livers. However, due to the experimental limitations of the used model, it remains unknown, whether this increase might also lead to a (clinically) relevant kidney or liver injury. Pretreatment of the allograft with ropivacaine did not have a significant impact on the inflammatory response measured in the kidneys and livers, although a significant reduction in the level of the proinflammatory cytokine TNF-α was previously observed in serum samples from these animals [6], which might indicate a multifactorial phenomenon leading to the observed pro-inflammatory signaling events. Unfortunately, we are not able to identify the exact mechanism, by which the inflammatory signaling cascade might be advanced throughout the process. Although several questions remain unanswered, taken together with the previously published assessment of the transplanted lungs [6], the results of the current study might lead to future experimental and clinical investigations ultimately leading to an improvement of patient outcome after transplantation.

DECLARATION OF COMPETING INTEREST

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TP reports financial support was provided by Hartmann Müller Foundation for Medical Research. TP reports a relationship with CSL Behring GmbH that includes speaking and lecture fees. TP reports a relationship with Edwards Lifesciences Corporation that includes speaking and lecture fees. All the other authors, declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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