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Article

ATRvD1 Attenuates Renal Tubulointerstitial Injury Induced by Albumin Overload in Sepsis-Surviving Mice

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Abstract: Current interventions are not effectives in preventing sepsis-induced acute kidney injury and its long-term outcomes or even after second renal insult. Therapeutic strategies using lipid mediators, as aspirin-triggered resolvin D1 (ATRvD1), can contribute for resolution of acute and chronic inflammation. In this study, we examined the potential effect of ATRvD1 on long-term kidney dysfunction after severe sepsis. Fifteen days after cecal ligation and puncture (CLP), sepsis-surviving BALB/c mice were subjected to a tubulointerstitial injury through intraperitoneal injections of bovine serum albumin (BSA) for 7 days, called subclinical acute kidney injury (subAKI) animal model. ATRvD1 treatment was performed right before BSA injections. On day 22 after CLP, urinary protein/creatinine ratio (UPC), histologic parameters, fibrosis, cellular infiltration, apoptosis, inflammatory markers levels, and mRNA expression were determined. ATRvD1 treatment mitigated tubulointerstitial injury by reducing the proteinuria excretion, UPC ratio, glomerular cell number and extracellular matrix deposition. Pro-fibrotic markers, as transforming growth factor β (TGFβ), type 3 collagen and metalloproteinase (MMP)-3 and -9 were reduced after ATRvD1 administration. Post-septic mice treated with ATRvD1 were protected from renal apoptosis and recruitment of $F4/80^+$ cells. Interleukin-1β (IL-1β) levels were increased in subAKI animal model, being attenuated by ATRvD1. Tumor necrosis factor-α (TNF-α), IL-10 and IL-4 mRNA expression was increased in the kidney of BSA-challenged post-septic mice and it was also reduced after ATRvD1. These results suggest that ATRvD1 protects the kidney against a second insult as BSA-induced tubulointerstitial injury and fibrosis by suppressing inflammatory and pro-fibrotic mediators in renal dysfunction after sepsis.

Keywords: sepsis; renal tubulointerstitial injury; resolvin; ATRvD1; inflammation; kidney;

1. Introduction

Sepsis is a life-threatening condition caused by dysregulated host responses and can lead to organ failure and death [1]. Prevention, early recognition of at-risk patients, and efficient supportive care have all contributed to a decline in the short-term mortality among septic patients [2–5]; however, long-term adverse outcomes are still evident after

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sepsis with impaired immune response in vital organs [6,7]. Following hospital discharge, one in five sepsis survivors is re-hospitalized due to infections or other acquired comorbidities [8]. In this regard, acute kidney injury (AKI) is one of the readmissions causes of septic patients who were discharged. Thus, kidney dysfunction is an important long-term outcome with a negative impact on quality of life [9]. To attenuate the long-term implications of renal failure, pharmacological strategies that promote kidney recovery are required.

While kidney dysfunction is strongly associated with mortality and other adverse outcomes in sepsis survivors, the inflammatory and immune pathways that lead to this phenomenon are still not clear [10]. To investigate these mechanisms, we previously verified renal damage in sepsis-surviving mice, being characterized by higher levels of urinary protein and creatinine (UPCr) ratio which was aggravated when post-septic mice were challenged with intraperitoneal bovine serum albumin (BSA) a model called subclinical acute kidney injury (subAKI). This model is characterized by an acute tubule-interstitial injury without changes in glomerular function and structure [11].

Specialized pro-resolving mediators (SPMs) derived from polyunsaturated fatty acids (PUFAs) potentially play an important role as pro-resolution and anti-inflammatory derivatives on inflammation. D- and E-series resolvins (RvD and RvE, respectively) are one of those mediators, they are biosynthesized by docosahexaenoic acid (DHA) and eicosapentenoic acid (EPA), respectively, via 15/5 lipoxygenase (LOX) or by aspirin-acetylated COX-2, which generates RvD epimers, called aspirin-triggered RvD (ATRvD), and 18*S*-RvE [12].

The potential role of SPMs in the resolution of sepsis has been explored. While the widespread systemic inflammation induced higher levels of leukotriene B_4 and prostaglandin E_2 in sepsis non-survivors, increased levels of RvE1 and RvD5 were associated with survival subjects [13]. Treatment of polymicrobial sepsis in mice with RvD1 and RvD2 reduced the inflammatory cytokines storm, neutrophil migration, enhances bacterial clearance, and improved the survival rate [14,15]. In addition, Zhuo et al demonstrated that RvD1 attenuated lung injury during systemic infection using murine cecal ligation and puncture (CLP) model [16]. Additionally, ATRvD1, a more potent and stable mediator than RvD1 [17], was able to reduce endotoxemia-induced AKI and to limit neutrophilic infiltration [18,19].

Based on the reported effects of RvD series, we postulated that ATRvD1 could attenuate long-term kidney dysfunction induced by a second insult after severe sepsis. Therefore, we evaluated the protective role of ATRvD1 on tubulointerstitial injury induced by BSA overload in a sepsis-surviving mouse model.

2. Results

2.1 *ATRvD1 Treatment Ameliorated BSA-induced Kidney Tubulointerstitial Injury in Post-Septic Mice*

Based on our previous data, sepsis-surviving mice already presented tubulointerstitial injury at days 7 and 14, which was aggravated after BSA challenge [11]. Along these lines, renal morphology was analyzed to determine whether ATRvD1 treatment was able to attenuate BSA-induced kidney insult after CLP, using a subAKI animal model (Figure S1). On day 22 post-surgery, CLP+BSA mice presented an increased kidney tubulointerstitial space (Figure 1A and 1C) and a higher ECM deposition (Figure 1B and 1D), compared to Sham+BSA mice. The administration of ATRvD1 attenuated these histomorphological changes (Figure 1A-D). The same feature was observed for glomerular cell number, ATRvD1 treatment reduced the increased number of cells in the glomeruli of CLP+BSA mice (Figure S2A-B). Though, a similar number of glomeruli was found in all groups (data not shown). In addition, there were no differences in renal morphology between the Sham+BSA and Sham+BSA+ATRvD1 mice.

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We then measured the proteinuria and urinary protein/creatinine ratio (UPCr) ratio. Proteinuria levels and the UPC ratio in 24 h were increased in CLP+BSA group, while ATRvD treatment reduced those parameters (Figure 1E-F). There was no difference in urine output among all groups (Figure S3).

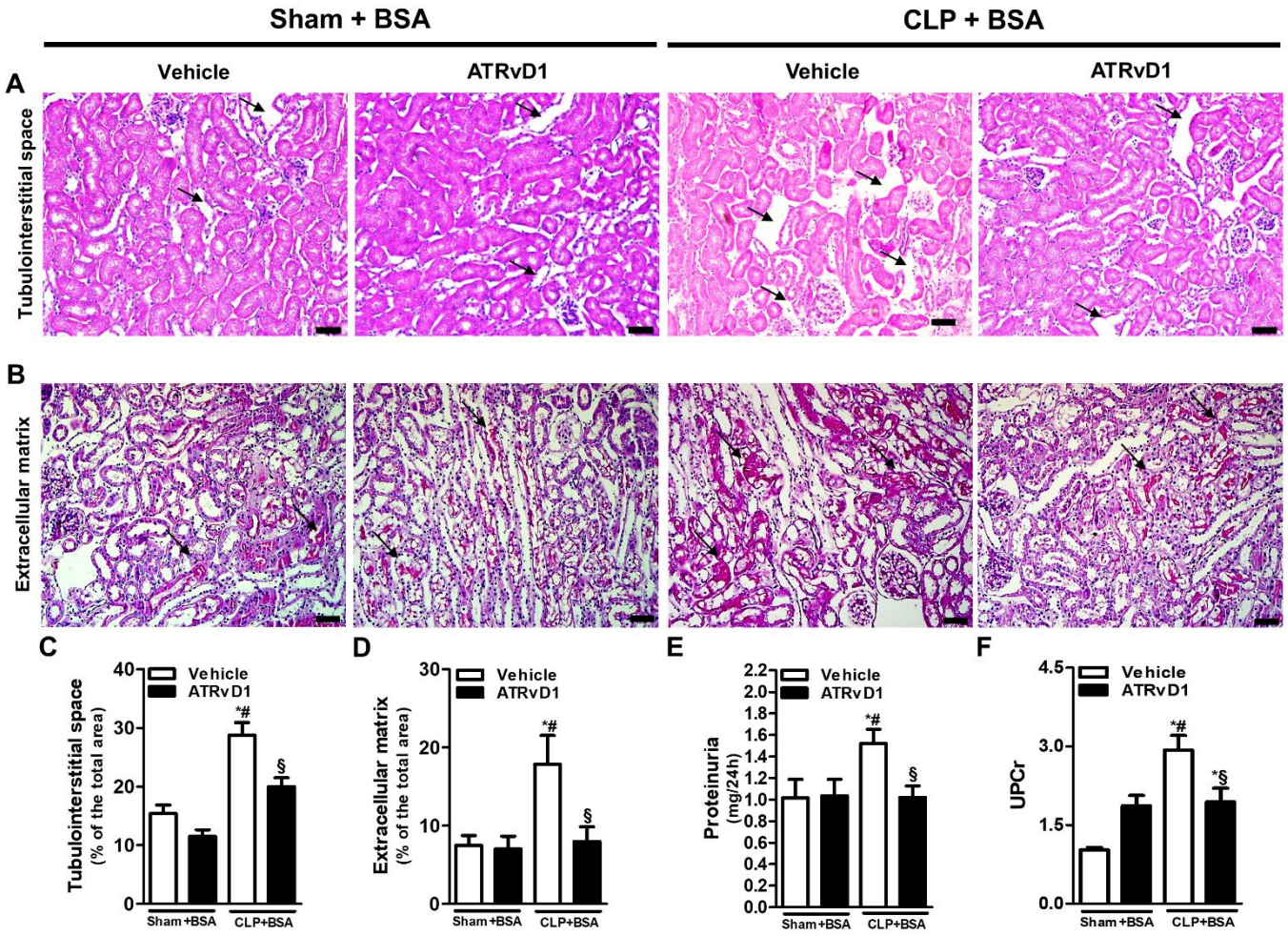

Figure 1. Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment Ameliorates BSA-Induced Kidney Tubulointerstitial Injury in Post-Septic Mice. Sham and cecal ligation and puncture (CLP)-surviving mice at day 15 were subjected to an intraperitoneal injection of 10g/kg bovine serum albumin (BSA) per day and treated with vehicle or ATRvD1 (i.v.) as described in the Material and Methods section. Kidneys were removed from animals on day 22 after surgery. (**A, C**) Tubulointerstitial space (20x) and quantitative analysis were assessed in tissue sections after hematoxylin and eosin staining; (**B, D**) Extracellular matrix deposition (20x) and quantitative analysis were assessed in tissue sections after Sirius Red staining. Images are representative of each group (n=3-5); (**E, F**) Renal function was evaluated by proteinuria and urinary protein/creatinine ratio (UPC) in the different experimental groups (n=3-10 for each experimental group). Graphics represent means ± SE. $^{*}p<0.05$ compared with non-treated Sham+BSA group; $^{\#}p<0.05$ compared with Sham+BSA+ATRvD1; $^{\S}p<0.05$ compared with non-treated CLP+BSA.

2.2 ATRvD1 Treatment Attenuated Collagen Deposition in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice

In order to evaluate the anti-fibrotic effect of ATRvD1 treatment we investigated the kidney collagen and fibronectin deposition. Figure 2A represents photomicrographs of histological slides of kidney tissue specimens stained with Picrosirius Red. An increased collagen deposition was observed in the CLP+BSA mice, whereas ATRvD1 treatment

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avoided it (Figure 2A and 2C). We then attempted to identify the type of collagen fibers in the renal tissue. Under polarized light, renal tissue from CLP+BSA mice contained predominantly green collagen fibers than that of CLP+BSA+ATRvD1 mice (Figure S4A), suggesting COL3 presence. Corroborating, we demonstrated more COL3 in the glomerular zones of CLP+BSA mice by immunohistochemical analysis compared to that of CLP+BSA+ATRvD1 mice (Figure 2B). Furthermore, the upregulated gene expression of COL3 and COL4 was inhibited by ATRvD1 treatment (Figure 2D-E). For fibronectin and COL1, similar gene expression (Figure S4B-C) and protein levels (data not shown) were detected among the groups.

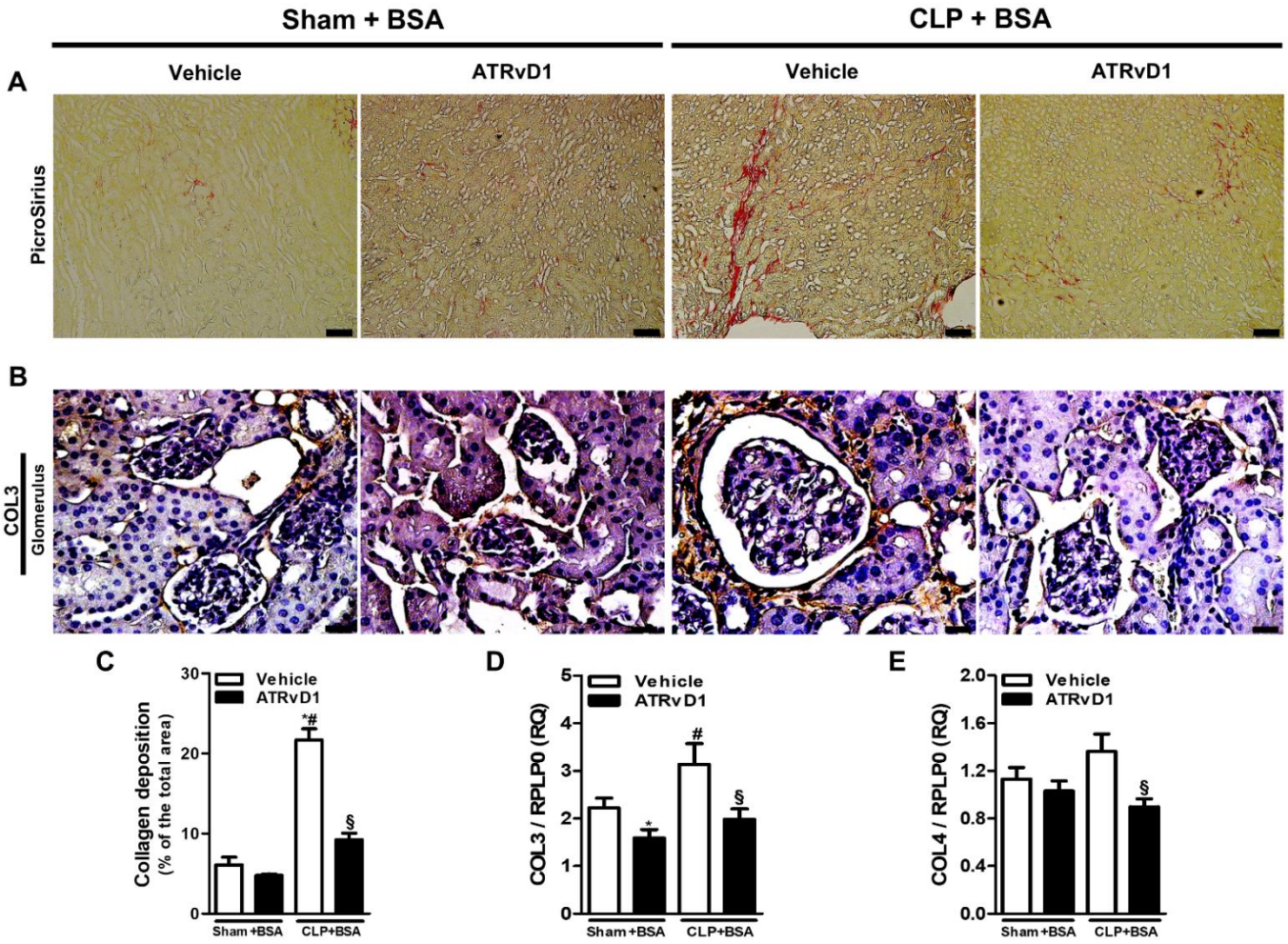

Figure 2. Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment Attenuates Collagen Deposition in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice. Sham and cecal ligation and puncture (CLP)-surviving mice at day 15 were subjected to i.p. injection of 10g/kg bovine serum albumin (BSA) per day and treated with vehicle or ATRvD1 (i.v.) as described in the Material and Methods section. Kidneys were removed from animals on day 22 after surgery. (**A,C**) Representative images of renal tissue sections after PicroSirius staining (10x) and quantitative analysis of collagen fibers deposition. (**B**) Representative images of glomerular areas of the renal tissue after immunohistochemical staining for COL3 (40x). Images are representative of each group (n=3-10). (**D, E**) Relative mRNA expression of COL3 and COL4 with RLP0 as endogenous control. (n=3-10 for each experimental group). Graphs represent means ± SE. $^{*}p<0.05$ compared with non-treated Sham+BSA group; $^{\#}p<0.05$ compared with Sham+BSA+ATRvD1; $^{\S}p<0.05$ compared with non-treated CLP+BSA.

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In response to injury, TGFβ is known to play a key role in fibrotic progression of the kidney. TGFβ was evaluated in the kidney tissue specimens using immunohistochemistry, ELISA, and RT-PCR methods. CLP+BSA group exhibited increased TGFβ expression in the cortical, medullar, and glomerular (Figure 3A-C) areas, compared to Sham+BSA groups. All parameters were reduced by ATRvD1 treatment to control levels (Figure 3A-C). Corroborating, TGFβ protein levels and mRNA expression were elevated in the kidney tissues of CLP+BSA group, and it was downregulated by ATRvD1 treatment (Figure 3D-E).

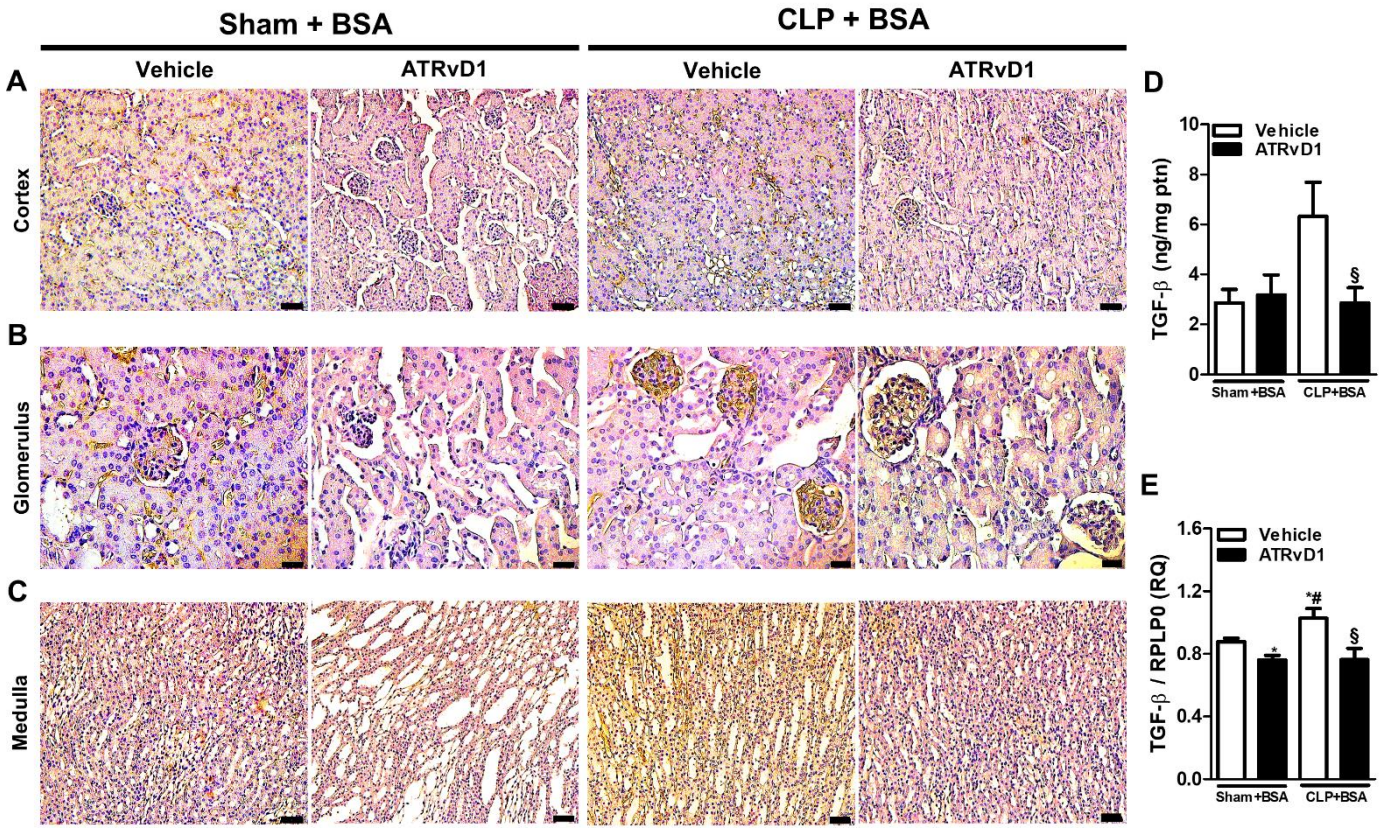

Figure 3. Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment Attenuates TGF-◎ expression in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice. Sham and cecal ligation and puncture (CLP)-surviving mice at day 15 were subjected to interperitoneally injection of 10g/kg bovine serum albumin (BSA) per day and treated with vehicle or ATRvD1 (i.v.) as described in the Material and Methods section. Kidneys were removed from animals on day 22 after surgery. (**A-C**) Representative images (n=3) of cortical (20x), glomerular (40x), and medullary (20x) areas of the renal tissue after immunohistochemical staining for TGFβ. (**D**) Renal TGFβ levels were measured using ELISA. (**E**) Relative mRNA expression of TGFβ with RLP0 as endogenous control. (n=3-10 for each experimental group). Graphs represent means ± SE. $^{*}p<0.05$ compared with non-treated Sham+BSA group; $^{\#}p<0.05$ compared with Sham+BSA+ATRvD1; $^{\S}p<0.05$ compared with non-treated CLP+BSA.

2.3 ATRvD1 Treatment Attenuated Inflammatory Markers in subAKI

Previous studies have demonstrated the accumulation of macrophages and increased apoptosis in renal tissue caused by BSA challenge [11, 20]. Therefore, we investigated whether ATRvD1 was able to reduce the number of F4/80^{+} cells and the amount of apoptosis in the renal cortex. Immunohistochemical staining of kidneys revealed that CLP+BSA

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mice treated with ATRvD1 exhibited a marked decrease in the number of F4/80⁺ cells (Figure 4A) and reduced apoptotic cells (Figure 4B).

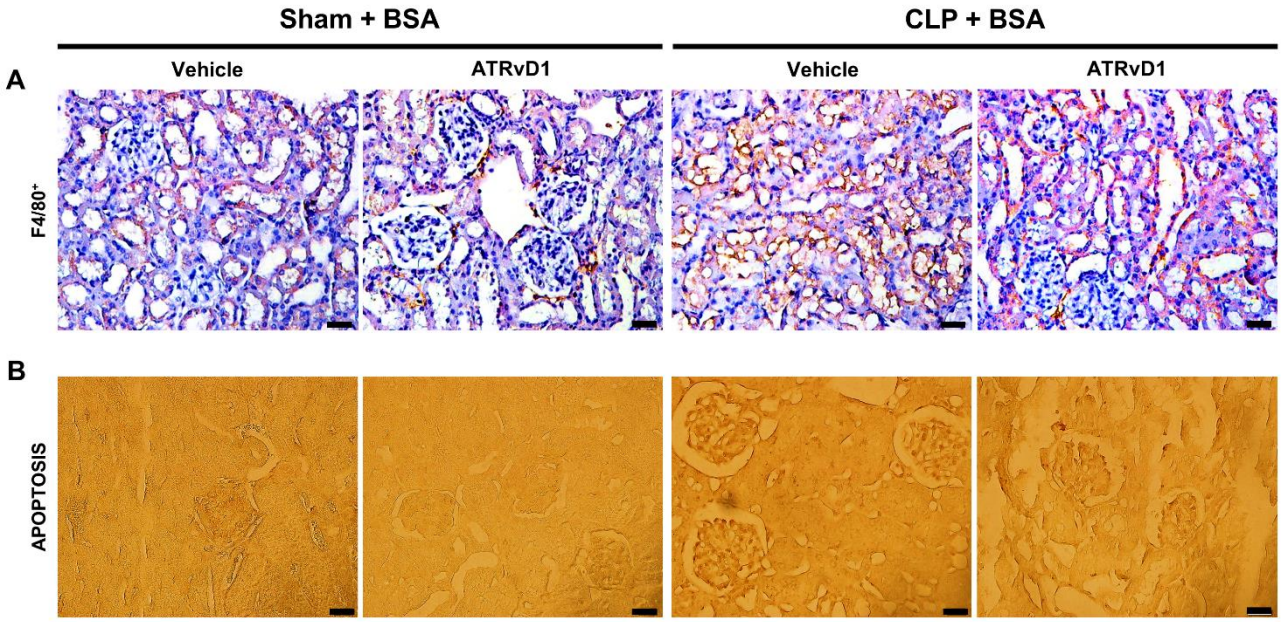

Figure 4. Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment Attenuates Inflammatory Markers in Subclinical Acute Kidney Injury. Sham and cecal ligation and puncture (CLP)-surviving mice at day 15 were subjected to interperitoneally injection of 10g/kg bovine serum albumin (BSA) per day and treated with vehicle or ATRvD1 (i.v.) as described in the Material and Methods section. Kidneys were removed from animals on day 22 after surgery. (A) Representative images of tissue renal sections after immunohistochemical staining for F4/80 (40x) as described in Material e Methods. (B) Representative images of apoptosis evaluation in tissue renal sections after TUNEL assay (40x). The data showed is representative of two independent experiments.

2.4 ATRvD1 Treatment Modulated MMP and Cytokine Production in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice

MMP-3 and MMP-9 perform pro-fibrotic roles by activating latent TGFβ [21]. Therefore, MMP-3 and MMP-9 levels were elevated in CLP+BSA group compared with those of Sham groups. Interestingly, ATRvD1 administration was able to reduce to the basal levels observed in the control groups (Figure 5A-B).

Pro-inflammatory and anti-inflammatory cytokine release was previously reported in the BSA-induced tubulointerstitial injury of post-septic mice [11]. IL-1β levels was increased in the CLP+BSA group compared to Sham groups, and it was abolished by ATRvD1 treatment (Figure 5C). Despite the fact that no statistical differences were observed between CLP+BSA and Sham groups in regard of TNF-α, IL-4, IL-6, IL-10, and CCL2 protein levels, it was observed greater amounts of these cytokines in the CLP+BSA group. Still, ATRvD1 therapy was able to reduce TNF-α, IL-10, and CCL2 levels, but not IL-4 and IL-6 levels (Figure S5A-E). In addition, the upregulated mRNA expressions of TNF-α, IL-4, and IL-10 in CLP+BSA group were significantly reduced by ATRvD1 therapy (Figure 5D–F).

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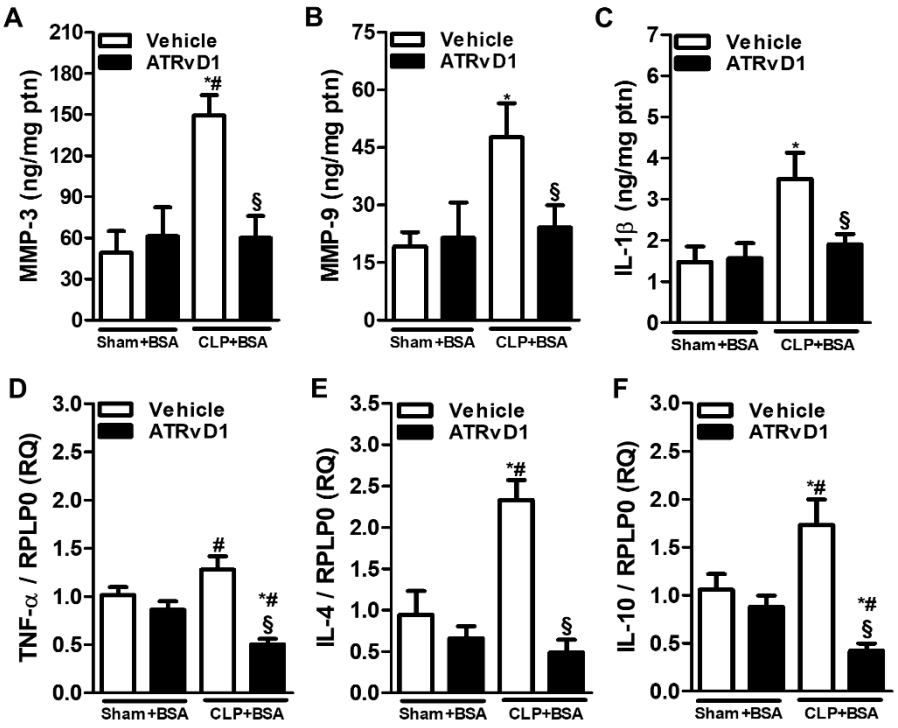

Figure 5. Matrix Metalloproteinases and Cytokine Production are Modulated by Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice. Sham and cecal ligation and puncture (CLP)-surviving mice at day 15 were subjected to interperitoneally injection of 10g/kg bovine serum albumin (BSA) per day and treated with vehicle or ATRvD1 (i.v.) as described in the Material and Methods section. Kidneys were removed from animals on day 22 after surgery, homogenized, and processed as described in Material and Methods. (**A-C)** MMP-3, MMP-9, and IL-1β levels were determined by ELISA. (**D-F)** Relative mRNA expression of TNF-α, IL-4 and IL-10 with RLP0 as endogenous control (n=3-10 for each experimental group). Graphs represent means ± SE. $^{*}p<0.05$ compared with non-treated Sham+BSA group; $^{\#}p<0.05$ compared with Sham+BSA+ATRvD1; $^{\S}p<0.05$ compared with non-treated CLP+BSA.

3. Discussion

Long-term dysfunction outcomes in sepsis-survivors are potentially linked to progressive impairment of quality of life and increased mortality [22]. Therapeutic strategies for persistent kidney dysfunction and increased pre-existing renal disorders after sepsis are limited. Our group has been engaged in investigating the recovery of homeostasis following long-term outcomes after sepsis [23].

Focusing on renal treatment for sepsis survivors, we used a subAKI model to investigate the potential effect of ATRvD1 on kidney dysfunction in sepsis-surviving mice. In our previous data, BSA challenge leads to inflammatory cytokine release and immune dysfunction in the cortical and medullary areas, which aggravates tubule damage and interstitial inflammation triggered by sepsis [11]. In addition, alterations in the albumin reabsorption machinery and changes in collagen deposition occur during this process [24]. We report here that ATRvD1 treatment reduced proteinuria excretion, UPC ratio, glomerular cell number and ECM deposition. ATRvD1 also attenuated inflammatory cytokines release and their mRNA expression, as well as cellular infiltration into kidney tissue of sepsis-surviving mice.

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Tubular damage and renal fibrosis have been reported in AKI experimental model [25], including albumin overload [20]. While Portella et al did not relate fibrosis and tubulointerstitial space alterations *in situ* [11], we verified that tubulointerstitial damage and renal fibrosis progression were reverted with ATRvD1 treatment. Our findings are supported by the ability of RvD and RvE in reducing interstitial fibrosis and myofibroblast proliferation in the kidney [26, 27]. In addition to reducing kidney fibrosis, both omega-3 supplementation (which increases endogenous renal levels of SPMs) and RvD1 attenuated tubulointerstitial injury and proteinuria [28, 29]. ATRvD1 also ameliorated endotoxemic renal failure in mice [18, 30].

TGFβ activates pro-fibrotic pathways, leading to ECM deposition. BSA overload induces high TGFβ levels, which correlates with the renal damage [20, 24]. Moreover, COL3 and COL4 are normally expressed in the kidney and are increased during fibrosis [31, 32]. In our study, ATRvD1 was able to counteract elevated TGFβ levels and mRNA expression stimulated by BSA overload. In line with this mechanism, Zheng et al demonstrated that RvD1 acts reducing TGFβ-induced collagen production [33]. In a unilateral ureteric obstruction (UUO) model, RvD1 and RvE1 inhibited COL1, COL3 and COL4 accumulation [27, 34]. Regarding decreased TGFβ in our study, RvD1 and ATRvD1 are potent regulators of TGFβ expression and production in acute and chronic inflammation [35-37]. In sum, these results strongly suggest that ATRvD1 plays pro-resolving actions in reducing profibrotic mediators, including ECM secretion, through TGFβ suppression.

MMP-9, activated by MMP-3, may be required for the release of active TGFβ [38, 39]. MMPs can be seen constitutively in tubules and glomerulus, however its expression is increased in AKI [32]. In our model, a possible mechanism for reducing TGFβ is the attenuation of MMP-3 and MMP-9 triggered by ATRvD1 treatment. Similarly, Posso et al demonstrated that ATRvD1 was able to downregulate MMP-3 expression in an emphysema model [40]. However, the effects of resolvins in the regulation of MMPs in renal disorders remain to be clarified.

Post-septic mice challenged with BSA present an immune dysfunction and inflammatory processes [11]. Consistent with those findings, we observed that ATRvD1 treatment attenuated inflammatory cytokine release, as well as the mRNA expression. There is evidence that RvD1 and RvD2 regulates pro- and anti-inflammatory cytokines in sepsis models [14–16]. The contribution of TNF-α to renal damage was confirmed using TNFR1$^{-/-}$ in endotoxemia, which prevents inflammation and apoptosis [41]. Corroborating, we addressed that apoptosis was attenuated by TNF-α reduction in mice treated with ATRvD1. Furthermore, based on evidence that IL-1β induces cellular recruitment and proteinuria and can mediate CCL2 and TNF-α expression in kidney, we believed that ATRvD1 treatment reduced F4/80^{+} cell recruitment following IL-1β attenuation [42, 43].

Our results suggest that the role of TGFβ in renal fibrosis could be addressed through Smad proteins, in which Smad3 induces ECM synthesis [44], but its deletion inhibits tubulointerstitial fibrosis [45]. In this way, RvD1 attenuated renal fibrosis through the inhibition of Smad2 linker phosphorylation in UUO model [34]. In the other hand, ATRvD1 activates Smad7, a key mediator that inhibits the TGFβ family and Smad2/3 signaling [46]. Other possible mechanism may be addressed to ALX/FPR2, one of the known RvD receptors. Interestingly, the RvD1-ALX/FPR2 axis inhibited TNFα receptor signaling and renal fibrosis [29]. Zhao et al described that activation of ALX/FPR2 in endotoxemia-induced AKI attenuated the inflammatory response [30]. In addition, mesangial cells proliferation can be inhibited through ALX/FPR2 mediation [47]. Along these lines, inhibition of ALX/FPR2 axis aggravated sepsis-induced kidney damage [48]. These reports allow to speculate that ATRvD1 exerts anti-inflammatory and pro-resolving actions by attenuating Smad 2/3 and activating Smad7 signaling, thereby suppressing TGFβ activation, MMP3 and MMP9 release, collagen deposition, cytokine production, cell recruitment, apoptosis, as well as tubule interstitial damage triggered by ATRvD1-ALX/FPR2 axis. Nevertheless, our findings require further investigation using our experimental model in order to explore the mechanisms of ATRvD1 restoration of kidney function.

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4. Materials and Methods

4.1 Animals

Male and female BALB/c mice (8–12 weeks old) were used in the study. The animals were obtained from the Brazilian National Institute of Cancer (Rio de Janeiro, Brazil) and Multidisciplinary Center for Biological Investigation (*UNICAMP, São Paulo, Brazil*). The mice were housed at a constant temperature of 25°C under a 12 h light/dark cycle with free access to food and water. All experiments were approved and performed in accordance with the ethical guidelines of the Institutional Animal Care Committee-CEUA in Federal University of Rio de Janeiro (protocol DFBCICB028 and protocol n° 130/16).

4.2 Cecal Ligation and Puncture (CLP) Model

Polymicrobial sepsis was induced by CLP as previously described [23]. Briefly, mice were i.p anesthetized with ketamine (112.5 mg/kg; Vetbrands, São Paulo, Brazil) and xylazine (7.5 mg/kg; Vetbrands, São Paulo, Brazil) and a 1-cm midline incision was made on ventral surface of the abdomen. The cecum was exposed, partial ligated below the ileocecal junction, and punctured twice (21G) before returning to the abdominal cavity. Sterile isotonic saline (1 mL) was administered subcutaneously after surgery. Sham-operated mice (Sham) were subjected to an identical laparotomy but without cecal ligation or puncture and were used as controls. All mice were i.p. treated with ertapenem antibiotic (75 mg/kg - Merck Research Laboratory, Whitehouse Station, NJ, USA) at 5, 24, 48, and 72 h post-surgery. Survival rates were determined daily for 15 d. CLP mice exhibited a 30–40% mortality rate after antibiotic treatment.

4.3 Subclinical Acute Kidney Injury and ATRvD1 Treatment

SubAKI model was induced in post-septic mice by intraperitoneal BSA injection as previously described [11]. The Sham and sepsis-surviving CLP mice were divided into four groups 15 d after surgery and received 10 g/kg BSA (i.p.) for seven consecutive days. The Sham and CLP groups received 50 µg/kg ATRvD1 (0.1% ethanol, i.v; Cayman Chemicals Co., Ann Arbor, Michigan, USA) at day 15 post-surgery followed by 5 µg/kg (0.01% ethanol) 20 min prior to BSA (Sigma-Aldrich) challenge for six days. Control groups received 0.1 or 0.01% ethanol (vehicle, i.v.) at the same days and volumes (100 µL) as the ATRvD1-treated groups. During treatment, the animals were housed in metabolic cages. At day 22, mice were anesthetized, and blood samples were collected by cardiac puncture. Kidneys were then immediately removed postmortem. One kidney from each animal was used for immunohistological studies while the other one was used for cytokine quantification by enzyme-linked immunosorbent assay (ELISA) and evaluation of the mRNA expression by quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis.

4.4 Renal Function Analysis

Mice were housed individually in metabolic cages and 24 h-urine, prior to euthanasia on day 22, was collected for volume and parameters analysis. Urine samples were centrifuged and stored at −20ºC. Protein and creatinine levels were determined by the pyragollol red method and the alkaline picrate method, using the Gold Analisa Kit 498M and Kit 335, respectively (Gold Analisa Diagnóstica, Belo Horizonte, Brazil).

4.5 Kidney Histology

Kidneys were collected, fixed at 4% buffered formalin, embedded in paraffin, and sectioned (5-µm thick). The tissue sections were stained with hematoxylin and eosin and assessed for glomeruli number, glomerular cell number, and interstitial space. Periodic acid Schiff and Picrosirius Red staining were used to evaluate the extracellular matrix (ECM) and collagen deposition, respectively. Data regarding interstitial space, ECM and collagen deposition were expressed as percentage of area stained by total tissue area. Cortical and medullary areas were photographed with an Olympus BX53 light microscope

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(Olympus, Tokyo, Japan) and analyzed using Image-Pro Plus software (Media Cybernetics, Rockville, MD, United States).

4.6 Immunohistochemistry analysis

Kidney sections were immunostained with antibodies (abs) against collagen type I (COL1; cat#sc-28654, Santa Cruz Biotechnology, Santa Cruz, CA) and transforming growth factor-β (TGFβ; cat#sc-146, Santa Cruz Biotechnology) at 1:150 dilutions; and abs against collagen type III (COL3; cat#ab7778, Abcam, Cambridge, MA, USA), F4/80 (cat#ab6640, Abcam), and fibronectin (cat#ab2413, Abcam) at 1:300 dilutions. As secondary abs, we used HRP-labeled polyclonal anti-rabbit abs (cat#ALI4404, Biosource International, Camarillo, CA), anti-mouse abs (cat#AMI4404, Biosource International) and anti-rat abs (cat#18-4818-82, eBioscience, San Diego, CA) at 1:1000 dilutions. The antibody reaction products were observed with the chromogen 3,3′- diaminobenzidine tetrachloride (DAB) (Spring Bioscience, USA). To visualize apoptotic cells, tissue sections were prepared according to the protocol provided with the DeadEnd™ Colorimetric TUNEL Kit (Promega Corporation, Madison, WI, USA).

4.7 Cytokine and ECM Protein Gene Expression Analysis by RT-PCR

Kidney specimens were collected, and total RNA was extracted using TRIZOL reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis was performed using 1 mg of total RNA as template and an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Primers targeting the genes for interleukin (IL)-4, IL-10, TGFβ, tumor necrosis factor-α (TNF-α, COL1, COL3, COL4, and fibronectin were designed using Primer Express software version 3.0 (Applied Biosystems, Waltham, MA, USA) and synthesized by Invitrogen (Table S1). RT-PCR assay was performed using Power SYBR Green Master Mix and the relative quantification method using the $2^{-\Delta\Delta Ct}$ calculation. Mouse large ribosomal protein, P0 (RPLP0) gene, was used as an endogenous control. Results were analyzed using StepOne Software v2.3 (Applied Biosystems, Waltham, MA, USA).

4.8 ELISA

Kidney levels of chemokine C-C motif ligand 2 (CCL2), IL-1β, IL-4, IL-6, IL-10, matrix metalloproteinase (MMP)-3, MMP-9, TGFβ, and TNF-α were measured using specific ELISA kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. Results were expressed as pg/mg of total protein.

4.10 Statistical Analysis

Differences in data between groups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test or a two-tailed unpaired Student's test. For all analyses, data were expressed as mean ± SEM. GraphPad Prism 5 Statistical Software Package (GraphPad Software, La Jolla, CA, USA) was used for all statistical analyses. P-value < 0.05 was considered statistically significant.

5. Conclusions

To successfully manage the long-term outcomes of sepsis, such as kidney failure, it is essential to identify new effective therapies. Thus, we proposed that ATRvD1 administration attenuates kidney tubulointerstitial damage and negatively modulates inflammatory mediators, cell apoptosis and cell infiltration intrinsically linked to fibrosis of sepsis-surviving mice subjected to a SubAKI model.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Subclinical Acute Kidney Injury model in sepsis-surviving mice and ATRvD1 Treatment., Figure S2: Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment Reduces Glomerular Cell Number in BSA-Induced Kidney Tubulointerstitial Injury in Post-Septic Mice., Figure S3: Aspirin-Triggered

Resolvin D1 (ATRvD1) Treatment Ameliorates BSA-Induced Kidney Tubulointerstitial Injury in Post-Septic Mice., Figure S4: Collagen Deposition and Extracellular Matrix Expression After Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment in BSA-Induced Kidney Tubulointerstitial Injury in Post-Septic Mice. Figure S5: Cytokine Production After Aspirin-Triggered Resolvin D1 (ATRvD1) Treatment in BSA-Induced Tubulointerstitial Injury in Post-Septic Mice. Table S1: Sequence primers used for Real Time PCR.

Author Contributions: J.B.N.F.S.: conceptualization, formal analysis, investigation, methodology, collected, analyzed the data, writing–original draft, writing-review and editing. T.B.B.C. and C.P.: formal analysis, collected, analyzed the data for this work and writing–review and editing. R.F.G.: collected and analyzed the data for this work. F.A.S. and K.S.C.: performed and analyzed all mRNA expression data and writing–review and editing. C.C.N.: resources, visualization, writing–review and editing. J.S.N.: conceived the project, visualization, supervision, writing–review and editing. C.F.B.: conceived the project, formal analysis, investigation, methodology, project administration, resources, funding acquisition, visualization, supervision, writing–original draft, writing–review and editing.

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Institutional Review Board Statement: This study was approved and performed in accordance with the ethical guidelines of the Institutional Animal Care Committee-CEUA in Federal University of Rio de Janeiro (protocol DFBCICB028 and protocol n° 130/16).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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References

1. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.D.; Coopersmith, C.M.; Hotchkiss, R.S.; Levy, M.M.; Marshall, J.C.; Martin, G.S.; Opal, S.M.; Rubenfeld, G.D.; van der Poll, T.; Vincent, J.L.; Angus, D.C. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **2016**, 315, 801-10. doi: 10.1001/jama.2016.0287.
2. Álvaro-Meca, A.; Jiménez-Sousa, M.A.; Micheloud, D.; Sánchez-Lopez, A.; Heredia-Rodríguez, M.; Tamayo, E.; Resino, S.; Group of Biomedical Research in Critical Care Medicine (BioCritic). Epidemiological trends of sepsis in the twenty-first century (2000–2013): an analysis of incidence, mortality, and associated costs in Spain. *Popul Health Metrics* **2018**, 16, 4. doi: 10.1186/s12963-018-0160-x.
3. Lu, Y.; Zhang, H.; Teng, F.; Xia, W.J.; Sun, G.X.; Wen, A.Q. Early Goal-Directed Therapy in Severe Sepsis and Septic Shock: A Meta-Analysis and Trial Sequential Analysis of Randomized Controlled Trials. *J Intensive Care Med* **2018**, 33, 296-309. doi: 10.1177/0885066616671710.
4. Gyawali, B.; Ramakrishna, K.; Dhamoon, A.S. Sepsis: The evolution in definition, pathophysiology, and management. *SAGE Open Med* **2019**, 7, 1-13. doi: 10.1177/2050312119835043.
5. Luhr, R.; Cao, Y.; Söderquist, B.; Cajander, S. Trends in sepsis mortality over time in randomised sepsis trials: a systematic literature review and meta-analysis of mortality in the control arm, 2002–2016. *Crit Care* **2019**, 23, 1-9. doi: 10.1186/s13054-019-2528-0.
6. Winters, B.D.; Eberlein, M.; Leung, J.; Needham, D.M.; Pronovost, P.J.; Sevransky, J.E: Long-term mortality and quality of life in sepsis: a systematic review. *Crit Care Med* **2010**, 38, 1276-83. doi: 10.1097/CCM.0b013e3181d8cc1d.
7. Schuler, A.; Wulf, D.A.; Lu, Y.; Iwashyna, T.J.; Escobar, G.J.; Shah, N.H.; Liu, V.X: The Impact of Acute Organ Dysfunction on Long-Term Survival in Sepsis. *Crit Care Med* **2018**, 46, 843-849. doi: 10.1097/CCM.0000000000003023.

8. Shankar-Hari, M.; Saha, R.; Wilson, J.; Prescott, H.C.; Harrison, D.; Rowan, K.; Rubenfeld, G.D.; Adhikari, N.K.J. Rate and risk factors for rehospitalisation in sepsis survivors: systematic review and meta-analysis. *Intensive Care Med* **2020**, 46, 619-636. doi: 10.1007/s00134-019-05908-3.
9. Prescott, H.C.; Langa, K.M.; Iwashyna, T.J. Readmission diagnoses after hospitalization for severe sepsis and other acute medical conditions. *JAMA* **2015**, 313, 1055–7. doi: 10.1001/jama.2015.1410.
10. Neyra, J.A.; Mescia, F.; Li, X.; Adams-Huet, B.; Yessayan, L.; Yee, J.; Toto, R.D.; Moe, O.W. Impact of acute kidney injury and CKD on adverse outcomes in critically ill septic patients. *Kidney Int Rep* **2018**; 3(6):1344-53. doi: 10.1016/j.ekir.2018.07.016.
11. Portella, V.G.; Silva-Filho, J.L.; Landgraf, S.S.; Rico, T.B.; Vieira, M.A.; Takiya, C.M.; Souza, M.C.; Henriques, M.G.; Canetti, C.; Pinheiro, A.A.; Benjamim, C.F.; Caruso-Neves, C. Sepsis-surviving mice are more susceptible to a secondary kidney insult. *Crit Care Med* **2013**, 41, 1056-68. doi: 10.1097/CCM.0b013e3182746696.
12. Serhan, C.N.; Chiang, N.; Dalli, J.; Levy, B.D. Lipid mediators in the resolution of inflammation. *Cold Spring Harb Perspect* Biol **2014**, 7, a016311. doi: 10.1101/cshperspect.a016311.
13. Dalli, J.; Colas, R.A.; Quintana, C.; Barragan-Bradford, D.; Hurwitz, S.; Levy, B.D.; Choi, A.M.; Serhan, C.N.; Baron, R.M. Human Sepsis Eicosanoid and Proresolving Lipid Mediator Temporal Profiles: Correlations With Survival and Clinical Outcomes. *Crit Care Med* **2017**, 45, 58-68. doi: 10.1097/CCM.0000000000002014.
14. Spite, M.; Norling, L.V.; Summers, L.; Yang, R.; Cooper, D.; Petasis, N.A.; Flower, R.J.; Perretti, M.; Serhan, C.N. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature* **2009**, 461, 1287-91. doi: 10.1038/nature08541.
15. Chen, F.; Fan, X.H.; Wu, Y.P.; Zhu, J.L.; Wang, F.; Bo, L.L.; Li, J.B.; Bao, R.; Deng, X.M. Resolvin D1 improves survival in experimental sepsis through reducing bacterial load and preventing excessive activation of inflammatory response. *Eur J Clin Microbiol Infect Dis* **2014**, 33, 457-64. doi: 10.1007/s10096-013-1978-6.
16. Zhuo, Y.; Zhang, S.; Li, C.; Yang, L.; Gao, H.; Wang, X. Resolvin D1 Promotes SIRT1 Expression to Counteract the Activation of STAT3 and NF-κB in Mice with Septic-Associated Lung Injury. *Inflammation* **2018**, 41,1762-1771. doi: 10.1007/s10753-018-0819-2.
17. Rogerio, A.P.; Haworth, O.; Croze, R.; Oh, S.F.; Uddin, M.; Carlo, T.; Pfeffer, M.A.; Priluck, R.; Serhan, C.N.; Levy, B.D. Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. *J Immunol* **2012**, 189:1983-91. doi: 10.4049/jimmunol.1101665.
18. Chen, J.; Shetty, S.; Zhang, P.; Gao, R.; Hu, Y.; Wang, S.; Li, Z.; Fu, J. Aspirin-triggered resolvin D1 down-regulates inflammatory responses and protects against endotoxin-induced acute kidney injury. *Toxicol Appl Pharmacol* **2014**, 277,118-23. doi: 10.1016/j.taap.2014.03.017.
19. Sok, M.C.P.; Tria, M.C.; Olingy, C.E.; San Emeterio, C.L.; Botchwey, E.A. Aspirin-Triggered Resolvin D1-modified materials promote the accumulation of pro-regenerative immune cell subsets and enhance vascular remodeling. *Acta Biomater* **2017**, 53,109-122. doi:10.1016/j.actbio.2017.02.020
20. Landgraf, S.S.; Silva, L.S.; Peruchetti, D.B.; Sirtoli, G.M.; Moraes-Santos, F.; Portella, V.G.; Silva-Filho, J.L.; Pinheiro, C.S.; Abreu, T.P.; Takiya, C.M.; Benjamin, C.F.; Pinheiro, A.A.; Canetti, C.; Caruso-Neves, C. 5-Lypoxygenase products are involved in renal tubulointerstitial injury induced by albumin overload in proximal tubules in mice. *PLoS One* **2014**, 9, e107549. doi: 10.1371/journal.pone.0107549
21. Giannandrea, M.; Parks, W.C: Diverse functions of matrix metalloproteinases during fibrosis. *Dis Model Mech* **2014**, 7, 193-203. doi: 10.1242/dmm.012062
22. Shankar-Hari, M.; Harrison, D.A.; Ferrando-Vivas, P.; Rubenfeld, G.D.; Rowan, K. Risk Factors at Index Hospitalization Associated With Longer-term Mortality in Adult Sepsis Survivors. *JAMA Netw Open* **2019**, 2, e194900. doi: 10.1001/jamanetworkopen.2019.4900.
23. Brogliato, A.R.; Antunes, C.A.; Carvalho, R.S.; Monteiro, A.P.; Tinoco, R.F.; Bozza, M.T.; Canetti, C.; Peters-Golden, M.; Kunkel, S.L.; Vianna-Jorge, R.; Benjamim, C.F. Ketoprofen impairs immunosuppression induced by severe sepsis and reveals an important role for prostaglandin E2. *Shock* **2012**, 38, 620-9. doi: 10.1097/SHK.0b013e318272ff8a.
24. Peruchetti, D.B.; Silva-Filho, J.L.; Silva-Aguiar, R.P.; Teixeira, D.E.; Takiya, C.M.; Souza, M.C.; Henriques, M.D.G.; Pinheiro, A.A.S.; Caruso-Neves, C. IL-4 Receptor α Chain Protects the Kidney Against Tubule-Interstitial Injury Induced by Albumin Overload. *Front Physiol* **2020**, 11. doi: 10.3389/fphys.2020.00172.
25. Katagiri, D.; Hamasaki, Y.; Doi, K.; Negishi, K.; Sugaya, T.; Nangaku, M.; Noiri, E. Interstitial renal fibrosis due to multiple cisplatin treatments is ameliorated by semicarbazide-sensitive amine oxidase inhibition. *Kidney Int* **2016**, 89, 374-85. doi: 10.1038/ki.2015.327.
26. Duffield, J.S.; Hong, S.; Vaidya, V.S.; Lu, Y.; Fredman, G.; Serhan, C.N.; Bonventre, J.V. Resolvin D series and protectin D1 mitigate acute kidney injury. *J Immunol* **2006**, 177, 5902-11. doi: 10.4049/jimmunol.177.9.5902.
27. Qu, X.; Zhang, X.; Yao, J.; Song, J.; Nikolic-Paterson, D.J.; Li, J. Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. *J Pathol* **2012**, 228, 506-19. doi: 10.1002/path.4050.
28. An, W.S.; Kim, H.J.; Cho, K.H.; Vaziri, N.D. Omega-3 fatty acid supplementation attenuates oxidative stress, inflammation, and tubulointerstitial fibrosis in the remnant kidney. *Am J Physiol Renal Physiol* **2009**; 297(4):F895-903. doi: 10.1152/ajprenal.00217.2009.

29. Zhang, X.; Qu, X.; Sun, Y.B.; Caruana, G.; Bertram, J.F.; Nikolic-Paterson, D.J.; Li, J. Resolvin D1 protects podocytes in adriamycin-induced nephropathy through modulation of 14-3-3β acetylation. *PLoS One* **2013**, 8, e67471. doi: 10.1371/journal.pone.0067471.
30. Zhao, Y.L.; Zhang, L.; Yang, Y.Y.; Tang, Y.; Zhou, J.J.; Feng, Y.Y.; Cui, T.L.; Liu, F.; Fu, P. Resolvin D1 Protects Lipopolysaccharide-induced Acute Kidney Injury by Down-regulating Nuclear Factor-kappa B Signal and Inhibiting Apoptosis. *Chin Med J (Engl)* **2016**, 129, 1100-7. doi: 10.4103/0366-6999.180517.
31. Genovese, F.; Manresa, A.A.; Leeming, D.J.; Karsdal, M.A.; Boor, P. The extracellular matrix in the kidney: a source of novel non-invasive biomarkers of kidney fibrosis? *Fibrogenesis Tissue Repair* **2014**, 7, 4. doi: 10.1186/1755-1536-7-4.
32. Parrish, A.R. Matrix Metalloproteinases in Kidney Disease: Role in Pathogenesis and Potential as a Therapeutic Target. *Prog Mol Biol Transl* Sci **2017**, 148, 31-65. doi: 10.1016/bs.pmbts.2017.03.001
33. Zheng, S.; Wang, Q.; D'Souza, V.; Bartis, D.; Dancer, R.; Parekh, D.; Gao, F.; Lian, Q.; Jin, S.; Thickett, D.R. ResolvinD1 stimulates epithelial wound repair and inhibits TGF-β-induced EMT whilst reducing fibroproliferation and collagen production. *Lab Invest* **2018**, 98, 130-140. doi: 10.1038/labinvest.2017.114.
34. Sun,Y.B.; Qu, X.; Li, X.; Nikolic-Paterson, D.J.; Li, J. Endothelial dysfunction exacerbates renal interstitial fibrosis through enhancing fibroblast Smad3 linker phosphorylation in the mouse obstructed kidney. *PLoS One* **2013**, 8, e84063. doi: 10.1371/journal.pone.0084063.
35. Yatomi, M.; Hisada, T.; Ishizuka, T.; Koga, Y.; Ono, A.; Kamide, Y.; Seki, K.; Aoki-Saito, H.; Tsurumaki, H.; Sunaga, N.; Kaira, K.; Dobashi, K.; Yamada, M.; Okajima, F. 17(R)-resolvin D1 ameliorates bleomycin-induced pulmonary fibrosis in mice. *Physiol Rep* **2015**, 3, e12628. doi: 10.14814/phy2.12628.
36. Saito, P.; Melo, C.P.B.; Martinez, R.M.; Fattori, V.; Cezar, T.L.C.; Pinto, I.C.; Bussmann, A.J.C.; Vignoli, J.A.; Georgetti, S.R.; Baracat, M.M.; Verri, W.A. Jr.; Casagrande, R. The Lipid Mediator Resolvin D1 Reduces the Skin Inflammation and Oxidative Stress Induced by UV Irradiation in Hairless Mice. *Front Pharmacol* **2018**, 9. doi: 10.3389/fphar.2018.01242. PMID: 30429790.
37. Horta, A.L.; Williams, T.; Han, B.; Ma, Y.; Menezes, A.P.J.; Tu, V.; Talvani, A.; Weiss, L.M.; Huang, H. Resolvin D1 Administration Is Beneficial in Trypanosoma cruzi Infection. *Infect Immun* **2020**, 88, e00052-20. doi: 10.1128/IAI.00052-20.
38. Lim, A.I.; Chan, L.Y.; Lai, K.N.; Tang, S.C.; Chow, C.W.; Lam, M.F.; Leung, J.C. Distinct role of matrix metalloproteinase-3 in kidney injury molecule-1 shedding by kidney proximal tubular epithelial cells. *Int J Biochem Cell Biol* **2012**, 44, 1040-50. doi: 10.1016/j.biocel.2012.03.015.
39. Bai, X.; Li, Y.Y.; Zhang, H.Y.; Wang, F.; He, H.L.; Yao, J.C.; Liu, L.; Li, S.S. Role of matrix metalloproteinase-9 in transforming growth factor-β1-induced epithelial-mesenchymal transition in esophageal squamous cell carcinoma. *Onco Targets Ther* **2017**, 10, 2837-2847. doi: 10.2147/OTT.S134813.
40. Posso, S.V.; Quesnot, N.; Moraes, J.A.; Brito-Gitirana, L.; Kennedy-Feitosa, E.; Barroso, M.V.; Porto, L.C.; Lanzetti, M.; Valença, S.S. AT-RVD1 repairs mouse lung after cigarette smoke-induced emphysema via downregulation of oxidative stress by NRF2/KEAP1 pathway. *Int Immunopharmacol* **2018**, 56, 330-338. doi: 10.1016/j.intimp.2018.01.045.
41. Cunningham, P.N.; Dyanov, H.M.; Park, P.; Wang, J.; Newell, K.A.; Quigg, R.J. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. *J Immunol* **2002**, 168, 5817-23. doi: 10.4049/jimmunol.168.11.5817.
42. Rovin, B.H.; Lu, L.; Marsh, C.B. Lymphocytes induce monocyte chemoattractant protein-1 production by renal cells after Fc gamma receptor cross-linking: role of IL-1beta. *J Leukoc Biol* **2001**, 69, 435-439.
43. Timoshanko, J.R.; Kitching, A.R.; Iwakura, Y.; Holdsworth, S.R.; Tipping, P.G. Leukocyte-derived interleukin-1beta interacts with renal interleukin-1 receptor I to promote renal tumor necrosis factor and glomerular injury in murine crescentic glomerulonephritis. *Am J Pathol* **2004**, 164, 1967-77. doi: 10.1016/s0002-9440(10)63757-1.
44. Higgins, C.E.; Tang, J.; Mian, B.M.; Higgins, S.P.; Gifford, C.C.; Conti, D.J.; Meldrum, K.K.; Samarakoon, R.; Higgins, P.J. TGF-β1-p53 cooperativity regulates a profibrotic genomic program in the kidney: molecular mechanisms and clinical implications. *FASEB J* **2019**, 33, 10596-10606. doi: 10.1096/fj.201900943R.
45. Sato, M.; Muragaki, Y.; Saika, S.; Roberts, A.B.; Ooshima, A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *J Clin Invest* **2003**, 112, 1486-94. doi: 10.1172/JCI19270.
46. Shu, Y.; Liu, Y.; Li, X.; Cao, L.; Yuan, X.; Li, W.; Cao, Q. Aspirin-Triggered Resolvin D1 Inhibits TGF-β1-Induced EndMT through Increasing the Expression of Smad7 and Is Closely Related to Oxidative Stress. *Biomol Ther (Seoul)* **2016**, Mar 1;24(2):132-9. doi: 10.4062/biomolther.2015.088.
47. McMahon, B.; Mitchell, D.; Shattock, R.; Martin, F.; Brady, H.R.; Godson, C. Lipoxin, leukotriene, and PDGF receptors cross-talk to regulate mesangial cell proliferation. *FASEB J* **2002**, 16, 1817-9. doi: 10.1096/fj.02-0416fje.
48. Gobbetti, T.; Coldewey, S.M.; Chen, J.; McArthur, S.; le Faouder, P.; Cenac, N.; Flower, R.J.; Thiemermann, C.; Perretti, M. Non-redundant protective properties of FPR2/ALX in polymicrobial murine sepsis. *Proc Natl Acad Sci U S A* **2014**, 111, 18685-90. doi: 10.1073/pnas.1410938111.