Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Stat-3 Signaling Role in an Experimental Model of Nephropathy Induced by Doxorubicin.

Thabata Santos (thabata.oliveira@ufpr.br)
Universidade Federal do Paraná: Universidade Federal do Parana https://orcid.org/0000-0002-7770-8758

Gabriel Pereira
UFPR: Universidade Federal do Parana

Ricardo Perez
UFPR: Universidade Federal do Parana

Anna Coutinho
UFPR: Universidade Federal do Parana

Halisson Pereira
UFPR: Universidade Federal do Parana

Debora Silva
Universidade Federal da Fronteira Sul

Marcelo Lima
UFPR: Universidade Federal do Parana

Fernando Dias
UFPR: Universidade Federal do Parana

Danilo Almeida
UNIFESP: Universidade Federal de Sao Paulo

Rafael Pereira
UFPR: Universidade Federal do Parana https://orcid.org/0000-0002-6056-724X

Research Article

Keywords: Focal Segmental Glomerulosclerosis, stat-3, nephropathy and doxorubicin

Posted Date: February 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1303649/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Focal Segmental Glomerulosclerosis (FSGS) is one of the most frequent glomerulopathies, and is considered a public health problem worldwide. FSGS is characterized by glomerular loss mainly due to inflammation and collagen fibers deposition. STAT-3 is a transcription factor associated with cell differentiation, migration and proliferation. Its activation occurs in response to several growth factors and cytokines. In renal cells, STAT-3 has been related with disease progression. Considering this perspective, the present study evaluated the involvement of STAT-3 in an experimental model of FSGS induced by Doxorubicin (DOX). First, we described a novel FSGS model in Swiss mice that after DOX administration showed typical signs of kidney dysfunction like higher proteinuria. Since there were no studies of ADM nephropathy model in heterogeneous mice, we were the first to evaluate the model in this lineage. Control animals treated with STAT-3 inhibitor (STATTIC) presented lower levels of albumin/creatinine ratio, glycosuria e proteinuria while DOX-injected mice showed higher levels. After analyzing some molecules involved in the STAT-3 signaling pathway, it was observed that STAT-3 blockade decreased levels of STAT-3, IL-6 and IL-6R, which remained elevated in DOX-administrated mice. Moreover, we detected that SOCS-3 (a regulator of STAT family) was up-regulated only in STATTIC-treated mice. Finally, histopathological analyzes showed that DOX-treated group had a significant increase in a tubulointerstitial fibrosis and tubular necrosis, which were not identified in both control and STATTIC groups. Thus, our results indicate that STAT-3 can have an important role in experimental FSGS induced by DOX and further studies are encouraged.

Introduction

The Focal Segmental Glomerulosclerosis (FSGS) is one of the main causes of chronic kidney disease (CKD), being considered a public health problem worldwide [1–3]. In Brazil, FSGS correspond to 25% of total CKD cases and furthermore is the most frequent glomerulopathy [3]. This disease is characterized by the obstruction of glomerular capillaries, which is mainly due to collagen fibers deposition [4, 5]. FSGS has several etiologies that are followed by different clinical prognoses [4, 5]. Commonly, FSGS can progress to end stage of renal disease with necessity of renal replacement therapies, such as transplant and/or hemodialysis (HD) [6, 7]. Although HD is widely used as principal therapy, at the same time, it possesses high annual cost for the public health system and is also associated with high morbidity and mortality [6, 7]. In this context, the search for understanding internal molecular mechanisms involved in the FSGS physiopathology is necessary to explore more effective treatment options.

In experimental models, FSGS can be mimicked trough the administration of chemotherapeutic agents like Doxorubicin hydrochloride (DOX), and this model is known as Doxorubicin nephropathy (DN) [4, 8–10]. DN reproduces most sign of FSGS as podocyte foot processes effacement, which is frequently associated with proteinuria triggering the progression of renal lesions through different pathways [2, 4, 5, 11, 12]. One major related cellular mechanism are the induction of tubular chemokines expression and complement system activation, which contribute to fibrosis and infiltration of inflammatory interstitials cells (i.e., macrophages) [13]. In addition, CKD induced proteinuria is close associated with the activation

of STAT signaling pathways (signal transducer and activator of transcription) [14–17], where STAT-3 play a pivotal role during inflammatory processes [16, 18–21].

STAT-3 is a transcription factor associated with cell differentiation, migration and proliferation, and its activation occurs in response to several growth factors and cytokines [21–23]. In renal cells, STAT-3 cascade has been related with lesions and progression of some renal diseases [17, 20, 24]. Specifically, the role of STAT-3 in renal diseases has been evaluated in some studies, which have pointed to a possible participation in the progression of CKD through the modulation of interstitial fibrosis [25, 26]. Whilst STAT-3 already have been linked to renal diseases progression, the specific contribution of this pathway for FSGS evolution is poorly elucidated. Considering this perspective, this present study evaluated the involvement of STAT-3 signal in an experimental model of FSGS induced by Doxorubicin.

Materials And Methods

Animals

Male swiss mice (*Mus musculus*) with age of 8-10 weeks were obtained from the Biological Sciences Sector of the Federal University of Paraná. The animals were housed in collective boxes, containing five mice per box, with light/dark artificial cycle of 12 hours at a constant ambient temperature of about 20°C and with free water and food supplies. The Ethics Committee of the Federal University of Paraná-Biological Sciences Center (CEUA/BIO-UFPR) approved the procedures for the use of animals under the number 957.

Experimental design

In order to induce the renal injury model in heterogenous mice, it was used the chemotherapeutic agent Doxorubicin hydrochloride (DOX), popular known as Adriamycin (Adriblastina®, Pfizer™, Inc. New York, USA). 25 mg/kg of DOX was injected in the caudal vein following the protocol of Jeansson *et al* [27]. To inhibit the STAT-3 molecular signal, the STATTIC (Abcam ™, Inc., Cambridge, MA) was administered intravenously at 2 mg/kg daily during all experiments. The animals were divided into four groups: 1) control group; 2) DOX group (treated with DOX only); 3) DOX + STATTIC group (treated with DOX and STATTIC); 4) STATTIC group (treated with STATTIC only). After five days mice were euthanized and biological samples (serum and kidney) were collected (Figure 1).

Estimative of albuminuria

The albuminuria was estimated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 10% with correction by urine creatinine levels [28]. The method consists in the migration of charged particles under the influence of an electric field in a viscous matrix. Then, the urine was diluted and mixed with a buffer solution (with mercaptoethanol as reducing agent). After, the gel was stained with Coomassie blue and albuminuria quantification was performed on a General Electric® Amersham Imager 600 photo documenter.

Urinalysis with reagent strips

For uranalysis the UroAction 10® uranalysis kit from Labtest (Labtest Diagnostics S.A. MG, Brazil) was used. This reagent consists in strips for rapid and semi-quantitative determination of glucose, bilirubin, ketone bodies (ketoacetic acid), density, blood, pH, protein, urobilinogen, nitrite and leukocytes in urine by colorimetric method.

Histological analysis

Histological analyzes were performed using double-blind tests. Kidneys fragments were stained with hematoxylin and eosin (H&E) for quantification of glomerular, tubular lesions and interstitial fibrosis under an optical microscope. Twelve random fields of each slide were analyzed using a photographic camera coupled to the computer containing the Zeiss® AxionVision software (Carl Zeiss, Oberkochen, Germany). For each field a semi-quantitative score was considered: 0, when there were no alterations; mild when had present alterations about 10%; moderate for alterations from 10–50%; and intense when changes were more than 50%. The pathological parameters evaluated were acute tubular necrosis, cellular and intracellular vacuolization, tubular collapse and distension and presence of inflammatory cells. Tubulo-interstitial fibrosis was characterized by expansion of the cellular matrix with distortion, collapse and thickening of the basement membrane. Alterations were considered positive when detected in more than five cells per analyzed field.

Real time PCR

For quantitative real-time PCR (qPCR), total RNA was extracted from frozen kidneys (-80ºC) using PureZOL® (Bio Rad, California, USA). The cDNA was synthesized using the reverse transcriptase enzyme (Bio Rad, California, USA) and the qPCR was performed using SYBR Green® kit (Bio Rad, California, USA). We use HPRT as housekeeping gene and the results were analyzed by the relative quantification method according to the formula $10{,}000/2^{\Delta ct}$ [29]. The primers to quantify the mRNAs for STAT-3, IL-6, IL-6R and SOCS-3 were synthesized using Primer Express software (Applied Biosystems, USA) based on the known sequence of nitrogen bases described in GenBank. The sequences of primers are as follows:

- Stat3 forward 5’- GGATCGCTGAGGTACAACCC- 3’;
- Stat3 reverse 5’- GTCAGGGGTCTCGACTGTCT- 3’;
- IL-6 forward 5’- CCTCTGGTCTTCTGGAGTACC- 3’
- IL-6 reverse 5’- ACTCCTTCTGTCACTCCAGC- 3’
- IL-6 R forward 5’- AAGAGTGACTTCCAGGTGCC- 3’
- IL-6 R reverse 5’- GGTATCGAAGCTGGAACTGC- 3’
- SOCS3 forward 5’- GGGTGGCAAAGAAAAGAAG- 3’
- SOCS3 reverse 5’- GTTGAGCGTCAAGACCCAGT- 3’

Statistical analyzes

Data are reported as means (SEM). All data were initially tested for normality by the Kolmogorov–Smirnov test. For parametric variables, differences between 2 groups were evaluated by the unpaired t-student test and for non-parametric variables, the Mann-Whitney test was utilized. In the case of more than two groups, the one-way analysis of variance (ANOVA) analysis was performed. For samples that did not present normal distribution, was used analysis of variance of single-point ANOVA, by the Kruskal-Wallis test. In the case of difference, the groups were compared with Student-Newman-Keuls correction for parametric variables and Dunn's correction for non-parametric variables. Differences were considered significant if the p value was < 0.05. Statistical analyzes were performed using GraphPad Prism® 6 software (GraphPad Software, San Diego, California - USA).

Results

STAT-3 signaling pathway inhibition prevent kidney disfunction

To validate our experimental model of focal segmental glomerulosclerosis (FSGS), we firstly injected doxorubicin hydrochloride (DOX) in animals and observed typical renal parameters of chronic kidney diseases (CKD). After 5 days, an intense increase in albuminuria and significant weight loss were observed in the group treated with DOX than control group (Figure 2). After to assess the role of STAT3 signaling pathway in DOX-induced nephropathy, we treated animals with STAT-3 inhibitor, STATTIC (known to inhibit STAT-3 phosphorylation by blocking dimers formation and consequent STAT-3 activation). We observed a significant albuminuria index in DOX treated-groups when compared to control group (Figure 3-A). STATTIC treatment slightly attenuated the levels of albumin/creatinine ratio (Figure 3A). The urinalysis also showed lower levels of glycosuria and proteinuria in the group control and mice treated with STATTIC in comparison with DOX-injected animals (Figure 3B and 3C). STATTIC treatment alone did not had substantial effect for all analysis.

STAT-3 blockade regulates renal inflammatory cytokines expression

In order to understand the mechanism behind of renal protection during STAT-3 inhibition, we evaluated major renal pro-inflammatory cytokines which are predicted to be involved in FSGS progression [22, 24–26, 30, 31]. Initially, we verified that STATTIC administration promoted a reduction in STAT-3 mRNA expression (Figure 4A). After, we observed in STATTIC-treated group a down regulation of IL-6R mRNAs, despite the fact IL-6 mRNA was t affected by STATTIC administration, suggesting a not activation of an inflammation cascade usually reported in chronic kidney diseases [8, 9, 32] (Figure 4B and 4C). In contrast, only for DOX group, the expression of both IL-6 and its receptor remained elevated (Figure 4B and 4C).

Moreover, we analyzed SOCS-3 expression (a pivotal regulatory molecule) during this kidney inflammatory process induced by DOX. The result indicated that treatment with STAT-3 inhibitor up-regulated the SOCS-3 mRNA expression, considering that control and DOX group demonstrated reduced values (Figure 4D). Thus, it is possible suggests that STATTIC inhibit the STAT-3 signaling pathway by

two independent mechanisms: i) direct blocking of STAT-3 signal and ii) by activation of SOCS3 which also attenuate STAT-3 cascade.

Inhibition of STAT-3 cascade protect kidney from tissue injury

The histopathological analyzes of the kidneys showed that DOX-treated group had a significant increase in a tubulointerstitial fibrosis and tubular necrosis. The same alterations did not were identified in both control and STATTIC groups (Figure 5 and 6). These findings indicate that treatment with DOX effectively induces a severe nephropathy with dramatic alteration in renal architecture. On the other hand, the treatment with STAT-3 inhibitor significantly decreased or prevented the tubule-interstitial fibrosis and tubular necrosis. Finally, as consequence of FSGS progression is expected an exacerbated infiltrating process with classical inflammatory cells presence in renal tissues. This event only was observed in DOX-injected animals and STATTIC administration blocked this process (Figure 5). Since these histological parameters are important in the progression of DOX-induced nephropathy, the results reported here point up that inhibition of STAT-3 attenuates renal injury which is often present in this experimental model.

Discussion

The focal segmental glomerulosclerosis (FSGS) is a complex renal disease characterized by different kind of lesions with distinct progression and responses to treatments. The search for a representative experimental model is fundamental to deep understand the course of disease. Balb/c mice is the classic strain used for FSGS experimental model, however others strain as C57/black 6 was also adapted presenting similar dysfunctions to classic model [27]. Here, we were innovative and by first time, it was adapted a heterogeneous mouse strain for experimental FSGS, Swiss line, that importantly mimic the heterogenicity of FSGS patients, providing tools for more effective investigations [2, 4–6, 11, 12, 32–36]. We observed in our novel experimental model that treatment with DOX caused typical FSGS signs (higher proteinuria and weight loss) in mice representing a new FSGS model in the literature, since the model of DOX nephropathy has not been yet developed in this lineage yet.

Considering that FSGS was adapted in our Swiss mice, we decided to investigate the participation of STAT-3 in our model of nephropathy induced by DOX. Proteinuria, especially albuminuria, is one of the main signs found in the model of DOX-induced nephropathy. Albuminuria is due to alterations in the glomerular filtration barrier and oxidative damage in the podocytes caused by DOX [8] [9, 37]. In our study, significant albuminuria was observed in animals injected with DOX and treatment with STATTIC significantly decreased not only albuminuria but also glycosuria in these animals. In addition, we detected that STATTIC modulated STAT-3 levels and was possible to pointed out the STAT-3 signal molecule as an important marker for experimental FSGS progression [38].

In order to evaluated the dynamic of STAT-3 signaling, we searched for pro-inflammatory cytokines present in this pathway and that could be altered in our model. Initially, we verified elevated IL-6 levels in DOX-treated animals. In contrast, the inhibition of STAT-3 signal caused a dramatic reduction in its expression. Interleukin-6 has a pleiotropic action on several biological functions and can be secreted by

many cell types through stimuli such as infection, inflammation or cancer [39]. For instance, The secretion of IL-6 by monocytes and macrophages following the activation of Toll-like receptors is especially important in cases of inflammation [40]. Moreover, Hunter & Jones [41] emphasized that there is extensive literature showing that IL-6 modulates various aspects of the innate immune system, including hematopoiesis and neutrophil accumulation at sites of infection or trauma. Thus, it is possible to infer that DOX causes inflammation, with a possible infiltration of macrophages, which in our study can be represented by elevated index of inflammatory cells in renal tissues. These cells are responsible to increase of IL-6 mRNA expression that is frequently observed in patients with primary FSGS associated with cutaneous and systemic plasmacytosis [42], beyond other nephropathies, such as IgA nephropathy [43]. In line with this, it was also detected significant differences in IL-6 receptor gene (IL-6R) expression between the DOX-injected and STATTIC-treated animals. The blocked of IL-6/IL-6R axis by inhibition of STAT-3 suggests a possible modulation of inflammation with consequent preservation of renal tissue and function. Furthermore, we observed that the treatment with STAT-3 inhibitor resulted in great increase of cytokine signaling suppressor type 3 (SOCS-3). SOCS-3 down regulates the JAK/STAT signal by binding simultaneously to glycoprotein Gp130 (one subunit of the type I cytokine receptor within the IL-6 receptor family) and JAK (a non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway), blocking binding to the substrate [44]. Thus, these findings can be interpreted as a cellular response for modulate inflammation caused by STAT-3 activation.

Finally, is reported that renal inflammation can trigger renal fibrosis cascade and impair kidney function [45]. Then is expected that DOX-mediated inflammation may promote fibrosis in FSGS experimental model. According to this we observed that DOX-administrated mice demonstrated consistent signs of tubulointerstitial fibrosis and tubular necrosis which did not were observed in control animals e STATTIC-treated group. Tao *et al* [46] using microscopic and transcriptomic evidences also identified glomerular and tubulointerstitial STAT-3 activation in renal biopsy of FSGS patients. Additionally, Bienaimè [25] yet demonstrated an important role for STAT-3 in tubulointerstitial communication during chronic kidney disease. The authors reported that STAT-3 activation promotes accumulation of interstitial matrix and fibroblasts, leading to increasing of tubulointerstitial fibrosis, while the STAT-3 inactivation causes decrease in tubulointerstitial fibrosis. Thus, our results indicate that STAT-3 pathway possess an important role in experimental FSGS induced by DOX and may be an important molecule to be investigated in further studies.

Conclusion

In summary our study documents a new adapted experimental model of focal segmental glomerulosclerosis (FSGS) providing a new platform for study of chronic nephropathy. All together our findings also suggest that STAT-3 signaling pathway participate actively of FSGS progression and its inhibition protects mice from kidney disfunction and injury. Although more precise mechanism should be investigated in relation to central role of STAT-3 in FSGS progression (i.e., up/down stream associated molecules), we believe that STAT-3 may be a predicted therapeutic target with great potential in the treatment of experimental FSGS.

Declarations

Funding

Thabata Caroline de Oliveira Santos was supported by a grant of CAPES (Coordenação de aperfeiçoamento de pessoal de nível superior) - Brazil.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the development of the study.

Data availability

The manuscript has no associated data

Ethics approval

The Ethics Committee of the Federal University of Paraná-Biological Sciences Center (CEUA/BIO-UFPR) approved the procedures for the use of animals under the number 957.

References

1. Bahiense-Oliveira M et al (2004) Primary glomerular diseases in Brazil (1979-1999): is the frequency of focal and segmental glomerulosclerosis increasing? Clin Nephrol 61(2):90–97
2. D'Agati VD et al (2013) Association of histologic variants in FSGS clinical trial with presenting features and outcomes. Clin J Am Soc Nephrol 8(3):399–406
3. Polito MG, de Moura LA, Kirsztajn GM (2010) An overview on frequency of renal biopsy diagnosis in Brazil: clinical and pathological patterns based on 9,617 native kidney biopsies. Nephrol Dial Transplant 25(2):490–496
4. Angioi A, Pani A (2016) FSGS: from pathogenesis to the histological lesion. J Nephrol 29(4):517–523
5. Sprangers B, Meijers B, Appel G (2016) FSGS: Diagnosis and Diagnostic Work-Up. Biomed Res Int, p 4632768
6. Uffing A et al (2020) Recurrence of FSGS after Kidney Transplantation in Adults. Clin J Am Soc Nephrol 15(2):247–256
7. Lafayette RA (2020) Facing the Vexing Problem of Recurrent FSGS after Kidney Transplantation. Clin J Am Soc Nephrol 15(2):171–173

8. Lee VW, Harris DC (2011) Adriamycin nephropathy: a model of focal segmental glomerulosclerosis. Nephrology (Carlton) 16(1):30–38
9. Okuda S et al (1986) Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. Kidney Int 29(2):502–510
10. Xie L et al (2019) Micro-CT imaging and structural analysis of glomeruli in a model of Adriamycin-induced nephropathy. Am J Physiol Renal Physiol 316(1):F76–F89
11. Shakeel S et al (2013) Frequency and clinicopathological characteristics of variants of primary focal segmental glomerulosclerosis in adults presenting with nephrotic syndrome. J Nephropathol 2(1):28–35
12. Shakeel S, Mubarak M, Kazi JI (2014) Frequency and clinicopathological correlations of histopathological variants of idiopathic focal segmental glomerulosclerosis in nephrotic adolescents. J Pak Med Assoc 64(3):322–326
13. Abbate M, Zoja C, Remuzzi G (2006) How does proteinuria cause progressive renal damage? J Am Soc Nephrol 17(11):2974–2984
14. Li Y et al (2019) PTPN2 improved renal injury and fibrosis by suppressing STAT-induced inflammation in early diabetic nephropathy. J Cell Mol Med 23(6):4179–4195
15. Liu Q et al (2020) Ellagic Acid Ameliorates Renal Ischemic-Reperfusion Injury Through NOX4/JAK/STAT Signaling Pathway. Inflammation 43(1):298–309
16. Liu Y et al (2020) C-X-C motif chemokine receptor 4 aggravates renal fibrosis through activating JAK/STAT/GSK3beta/beta-catenin pathway. J Cell Mol Med 24(7):3837–3855
17. Tao J et al (2020) JAK-STAT Activity in Peripheral Blood Cells and Kidney Tissue in IgA Nephropathy. Clin J Am Soc Nephrol 15(7):973–982
18. Huang JS et al (2015) Cinnamaldehyde and nitric oxide attenuate advanced glycation end products-induced the Jak/STAT signaling in human renal tubular cells. J Cell Biochem 116(6):1028–1038
19. O'Leary R et al (2016) Macrophage-derived IL-6 contributes to ANG II-mediated angiotensinogen stimulation in renal proximal tubular cells. Am J Physiol Renal Physiol 310(10):F1000–F1007
20. Zhang B et al (2020) Interference with miR-210 Alleviated Renal Injury in Septic Rats by Inhibiting JAK-STAT Pathway. Inflammation
21. Gu Y, Mohammad IS, Liu Z (2020) Overview of the STAT-3 signaling pathway in cancer and the development of specific inhibitors. Oncol Lett 19(4):2585–2594
22. Abualsunun WA et al (2020) Essential role of STAT-3 dependent NF-kappaB activation on IL-6-mediated downregulation of hepatic transporters. Eur J Pharm Sci 143:105151
23. Yang YL et al (2020) Stat-3 signaling promotes cell proliferation and metastasis of gastric cancer through PDCD4 downregulation. Kaohsiung J Med Sci 36(4):244–249
24. Chuang PY, He JC (2010) JAK/STAT signaling in renal diseases. Kidney Int 78(3):231–234
25. Bienaime F et al (2016) Stat3 Controls Tubulointerstitial Communication during CKD. J Am Soc Nephrol 27(12):3690–3705

26. Pang M et al (2010) A novel STAT3 inhibitor, S3I-201, attenuates renal interstitial fibroblast activation and interstitial fibrosis in obstructive nephropathy. Kidney Int 78(3):257–268
27. Jeansson M et al (2009) Adriamycin alters glomerular endothelium to induce proteinuria. J Am Soc Nephrol 20(1):114–122
28. Cybulsky AV et al (2020) Podocyte injury and albuminuria in mice with podocyte-specific overexpression of the Ste20-like kinase, SLK. Am J Pathol 177(5):2290–2299
29. de Almeida DC et al (2016) A Regulatory miRNA-mRNA Network Is Associated with Tissue Repair Induced by Mesenchymal Stromal Cells in Acute Kidney Injury. Front Immunol 7:645
30. Ahmad SF et al (2017) STA-21, a STAT-3 inhibitor, attenuates the development and progression of inflammation in collagen antibody-induced arthritis. Immunobiology 222(2):206–217
31. Pedroza M et al (2016) STAT-3 contributes to pulmonary fibrosis through epithelial injury and fibroblast-myofibroblast differentiation. FASEB J 30(1):129–140
32. Fogo AB (2003) Animal models of FSGS: lessons for pathogenesis and treatment. Semin Nephrol 23(2):161–171
33. Campbell KN, He JC (2014) Can biomarkers of disease activity guide treatment in FSGS? Clin J Am Soc Nephrol 9(9):1507–1509
34. Cravedi P, Kopp JB, Remuzzi G (2013) Recent progress in the pathophysiology and treatment of FSGS recurrence. Am J Transplant 13(2):266–274
35. Korbet SM (2012) Treatment of primary FSGS in adults. J Am Soc Nephrol 23(11):1769–1776
36. Sethna CB, Gipson DS (2014) Treatment of FSGS in Children. Adv Chronic Kidney Dis 21(2):194–199
37. Pereira RL et al (2012) Invariant natural killer T cell agonist modulates experimental focal and segmental glomerulosclerosis. PLoS ONE 7(3):e32454
38. Scott RP, Quaggin SE (2015) Review series: The cell biology of renal filtration. J Cell Biol 209(2):199–210
39. Garbers C, Aparicio-Siegmund S, Rose-John S (2015) The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. Curr Opin Immunol 34:75–82
40. Calabrese LH, Rose-John S (2014) IL-6 biology: implications for clinical targeting in rheumatic disease. Nat Rev Rheumatol 10(12):720–727
41. Hunter CA, Jones SA (2015) IL-6 as a keystone cytokine in health and disease. Nat Immunol 16(5):448–457
42. Isobe S et al (2017) Focal segmental glomerulosclerosis associated with cutaneous and systemic plasmacytosis. CEN Case Rep 6(2):206–209
43. Yoshioka K et al (1993) In situ expression of cytokines in IgA nephritis. Kidney Int 44(4):825–833
44. Carow B, Rottenberg M (2014) SOCS3, a Major Regulator of Infection and Inflammation. Front Immunol 5:58
45. Meng XM (2019) Inflammatory Mediators and Renal Fibrosis. Adv Exp Med Biol 1165:381–406

46. Tao J et al (2018) JAK-STAT signaling is activated in the kidney and peripheral blood cells of patients with focal segmental glomerulosclerosis. Kidney Int 94(4):795–808

Figures

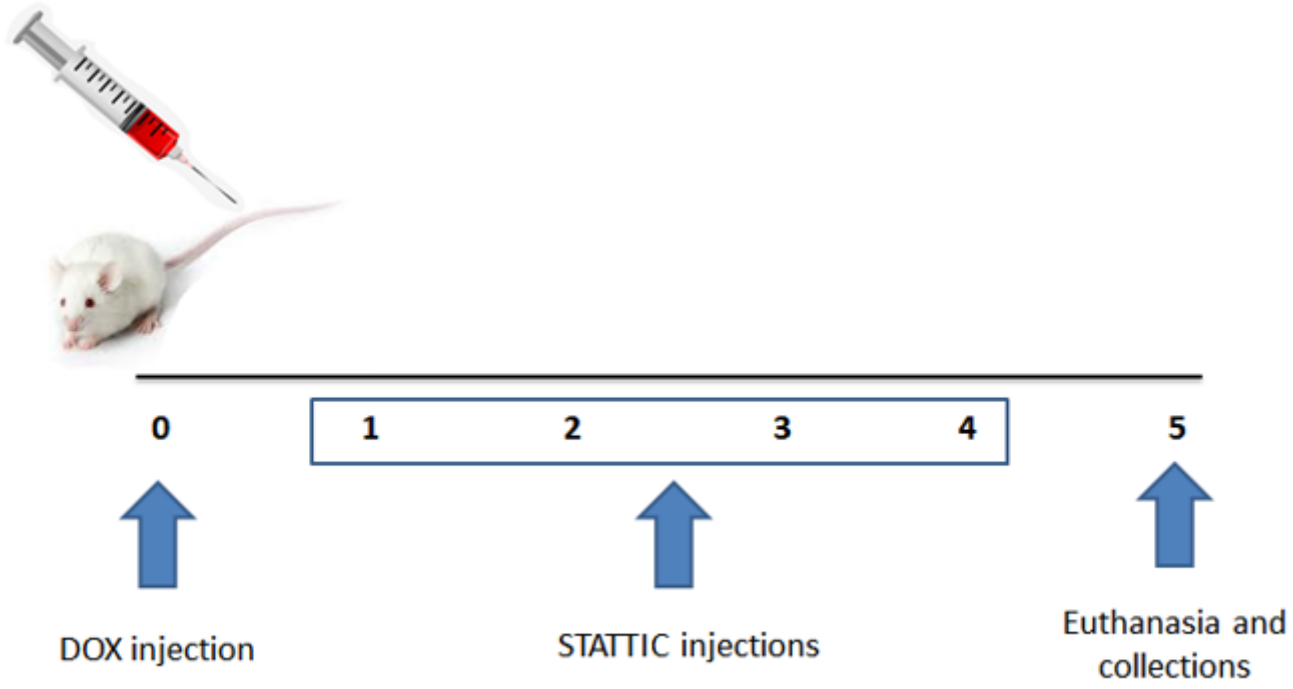

Figure 1

Experimental design of mice model of focal segmental glomerulosclerosis (FSGS). Swiss mice were injected with 25 mg/kg of Doxorubicin hydrochloride (DOX), popular known as Adriamycin. The experiment had duration of 5 days and STATTIC was injected daily until day 5 where blood and renal tissues were collected for several analysis

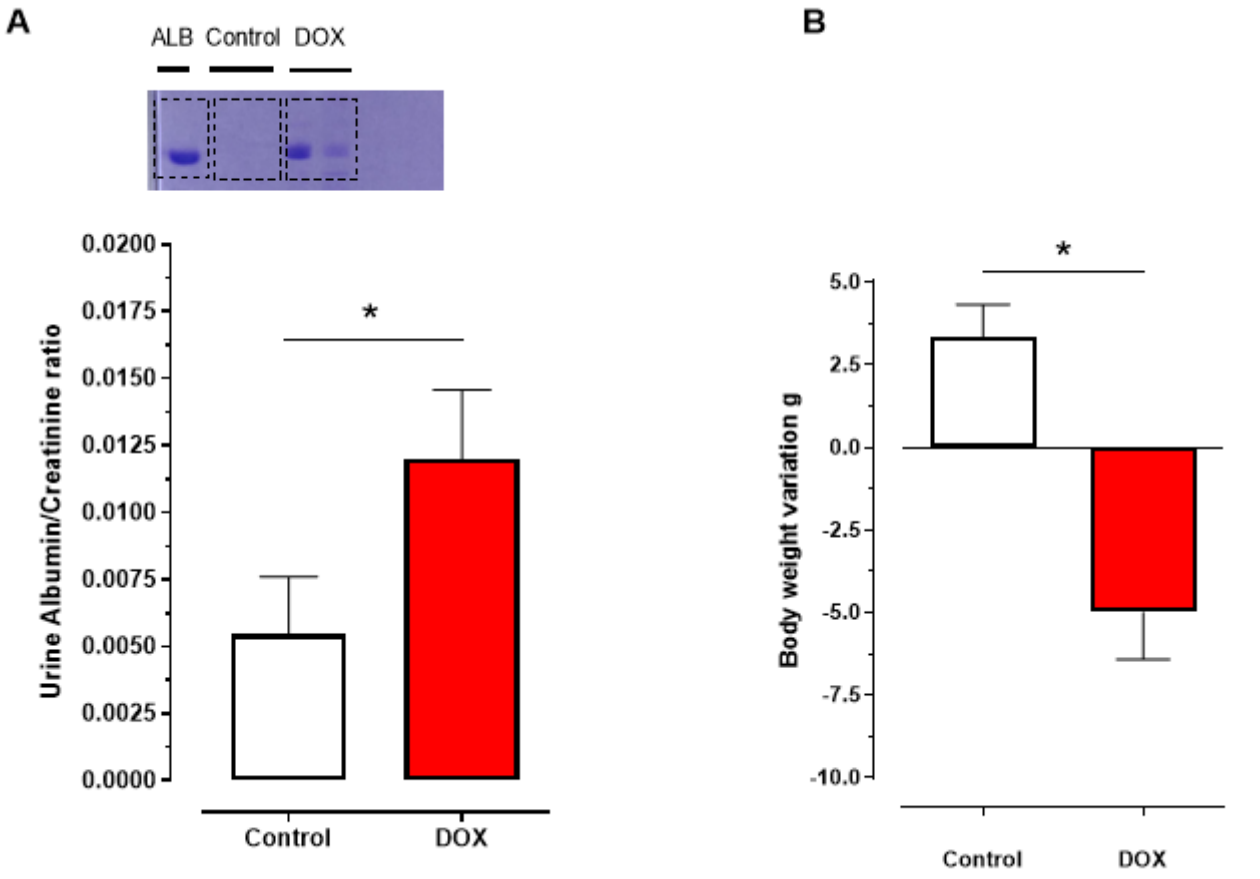

Figure 2

Evaluation of renal dysfunction after administration of Doxorubicin (DOX) in Swiss mice. 25 mg/kg of DOX was injected in mice and after 5 days was observed intense albuminuria (A) and weight loss (B). n=10 animals per study group, p<0.05. ALB=Urine albumin control

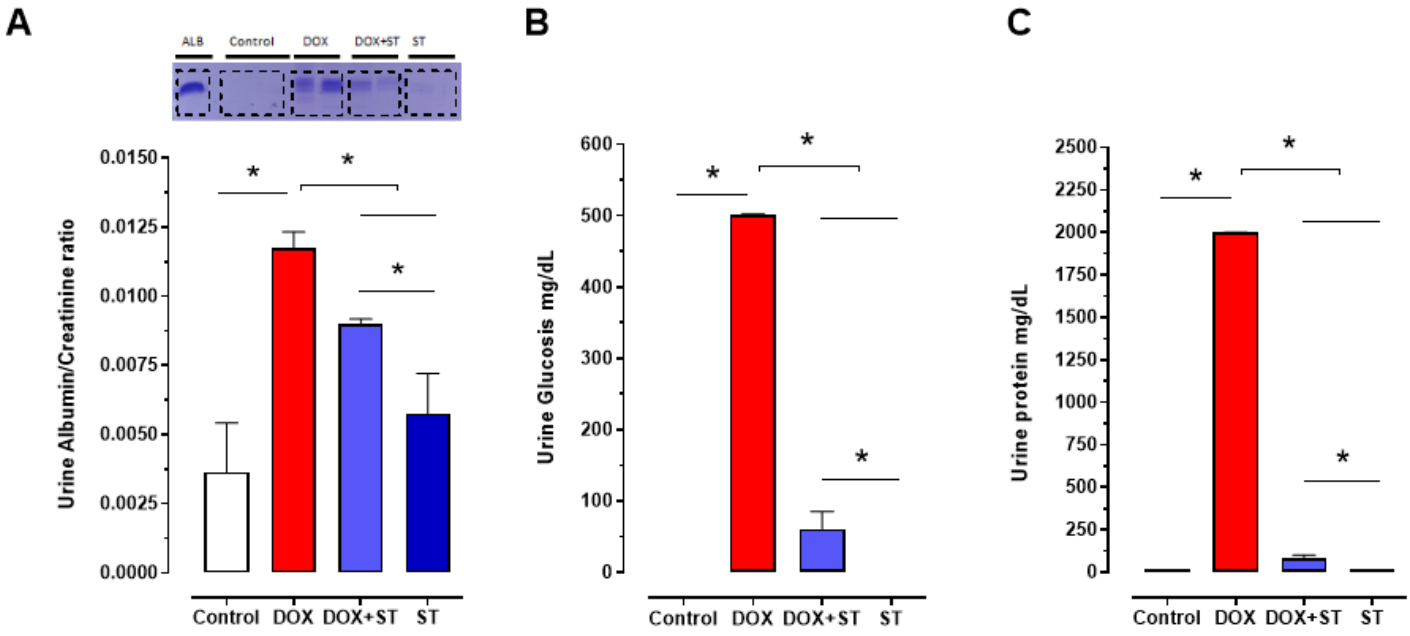

Figure 3

Inhibition of STAT-3 signal protects mice from renal disfunction. (A): SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 10% with correction by urine creatinine levels; (B) uranalysis of glucose; and (C) uranalysis of protein. Through urinalysis significant albuminuria differences were detected between the DOX group and the control group and between the DOX and DOX + STATTIC groups. The DOX group had significantly higher urinary glucose and protein concentration than the other groups ($p < 0.05$). n=10 animals per study group, $p<0.05$

Figure 4

Effect of inhibition of STAT-3 in pro-inflammatory and regulatory molecules. (A) mRNA expression by RT-PCR of STAT-3; (B) mRNA expression by RT-PCR of IL-6R; (C) mRNA expression by RT-PCR of IL-6; and (D) mRNA expression by RT-PCR of SOCS-3. STATTIC-treated groups showed significantly lower STAT-3 mRNA expression than the other groups. Groups treated with DOX showed significantly higher IL-6 and IL-6R mRNA levels than the other groups. SOCS-3 mRNA expression was significantly higher in the DOX + STATTIC group than in the control group and DOX group. The treatment only with STATTIC did not show representative effect. n=10 animals per study group, $p<0.05$

Figure 5

Histopathological analysis of the kidney's sections. (A) quantification of fibrosis score; (B) quantification of inflammatory cells infiltrate; and (C, D and E) histological sections of renal tissues stained with hematoxylin and eosin (H&E). Black arrows indicate structural derangements and alterations from Mild, Moderate and High levels indicated by C, D and E, respectively. Images are represented with 400X magnification

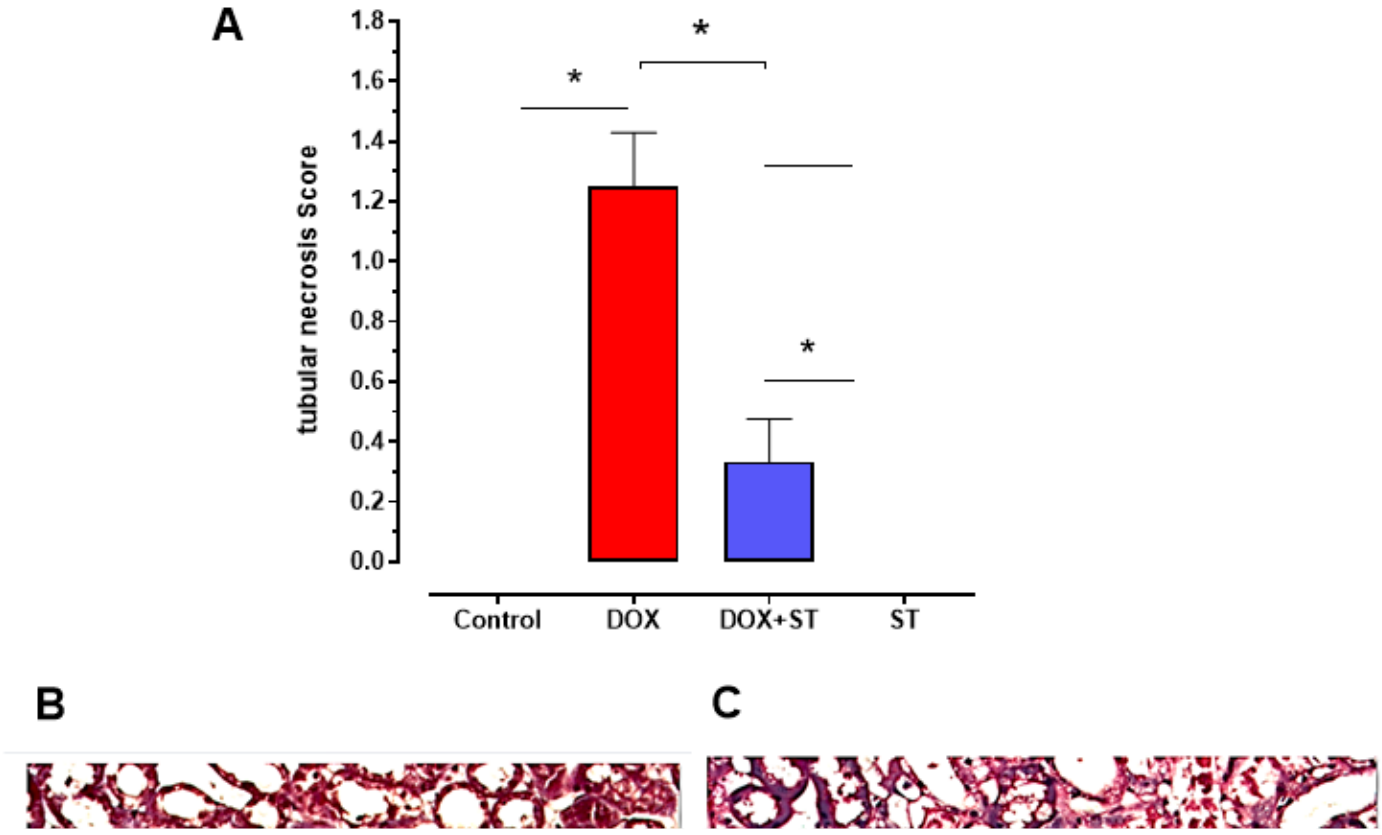

Figure 6

Histological analysis of tubular necrosis. (A) Quantification of tubular necrosis score; (B) representative image of health control kidneys; and (C) representative image of severely affected kidney by DOX infusion. Mice injected with DOX presented higher scores of tubular damages and the treatment with inhibitor of STAT-3 reduced this index. Black arrows indicate structural derangements and alterations. Images are represented with 400X magnification