# Splenectomy Attenuates the Course of Kidney Ischemia-Reperfusion Injury in Rats

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## ABSTRACT

Introduction. Renal ischemia-reperfusion injury (IRI) initiates inflammatory response with synthesis of free oxygen radicals, chemokines, and cytokines which attract neutrophils and monocytes, which then differentiate into macrophages and dendritic cells, activating adaptive immune response. The spleen is the main source of both monocytes and lymphocytes. The aim of this study was to assess whether splenectomy performed before or upon IRI affects post-ischemic and long-term renal function.

Methods. Two weeks after right nephrectomy, the left kidney pedicle was clamped for 45 minutes in 24 rats. After the clip insertion, the spleen was removed in 12 animals and the remaining 12 rats underwent sham splenectomy. In the second experiment, splenectomy ($n = 9$) or sham procedure ($n = 9$) was performed simultaneously with right nephrectomy, 2 weeks before left kidney ischemia. The excretory function of the kidney was evaluated 48 hours and 7 days after ischemia. In the experimental model of chronic renal failure, 14 days before right nephrectomy, the prolonged 90-minute ischemia was induced in 32 rats with simultaneous splenectomy ($n = 16$) or sham procedure ($n = 16$). In long-term observation, the renal function and mortality rate was evaluated.

Results. Kidney function preservation was superior in rats that underwent splenectomy together with renal ischemia when compared to controls. This was further expressed with a 2 times lower mortality rate in splenectomized animals in 6 months observation after prolonged renal ischemia. Renoprotective effect was not observed when splenectomy was performed 2 weeks before IRI.

Conclusions. The results suggest a detrimental influence of the spleen on the development of renal IRI.

RENAL ISCHEMIA-REPERFUSION INJURY (IRI) causes glomerular capillary endothelial dysfunction and tubular epithelial necrosis leading to ischemic acute kidney injury (AKI) with activation of inflammatory reaction mediated by increased cytokine and adhesion molecule expression, synthesis of reactive oxygen species and chemoattractants, leading to neutrophil, monocyte, and lymphocyte activation and infiltration [1]. As the part of innate immune system, macrophages play a crucial role in the inflammatory reaction and development of AKI through synthesis of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, IL-12, tumor necrosis factor alpha, and complement components [2]. Furthermore, macrophage-derived transforming growth factor beta promotes interstitial fibrosis leading ultimately to chronic renal failure [3]. Some of the infiltrating monocytes differentiate into antigen-presenting dendritic cells (DCs) [4]. Activation of DC expressed Toll-like receptors leads to stimulation of an adaptive immune response responsible for chronic renal failure and which, in the setting of a kidney allograft, is co-responsible for its chronic rejection.

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Heat shock proteins released during IRI, as well as components of necrotic cells act as endogenous ligands for Toll-like receptors, leading to DC activation with increased major histocompatibility complex II expression. Antigen-presenting cells migrating to the secondary lymphatic organs activate naive T lymphocytes with their differentiation into cytotoxic, as well as type 1 and 2 T helper cells [5,6]. These dependencies have been confirmed by observing kidney infiltration with CD4+ and CD8+ T cells 6 weeks after experimental 60-minute renal ischemia, as well as improved post-ischemic renal function in CD4+-deficient rats [7]. This phenomenon of interplay between innate and adaptive immune responses mediated, inter alia, by infiltrating monocytes and lymphocytes, may explain significant influences of IRI and the course of AKI on long-term renal function. The spleen is the main unit of the mononuclear phagocytic system (MPS) comprising monocytes, macrophages, and DCs. Furthermore, as the largest single lymphoid organ, it is the main reservoir and source of lymphocytes, and the predominant site of T cells activation. The aim of this study was to assess the influence of splenectomy (Splx) performed 2 weeks before or upon kidney ischemia on the course of ischemic AKI and long-term renal function in the rat.

## METHODS

Seventy-four male Sprague-Dawley rats (9-weeks old, weighing 250 to 300 g) were obtained from the Experimental Medicine Centre, Medical University of Silesia, Katowice, Poland. Animals received humane care in compliance with the "Principals of Laboratory Animal Care." All rats were provided with standard laboratory chow and water ad libitum in a temperature-controlled environment (21°C) with a 12-hour light-dark cycle. In the first experiment, 2 weeks after right nephrectomy, the left kidney pedicle was clamped for 45 minutes in 24 rats. After the clip insertion, the spleen was removed in 12 randomly chosen animals (group 1). The remaining 12 rats underwent sham splenectomy (group 2, control). In the second experiment including 18 rats, splenectomy (group 3, n = 9) or sham procedure (group 4, control, n = 9) was performed simultaneously with right nephrectomy, 2 weeks before solitary kidney ischemia. In the third experiment, 90-minute ischemia of the left kidney was performed, with simultaneous splenectomy (group 5, n = 16) or sham operation (group 6, control, n = 16). The right nephrectomy was performed 2 weeks later. Forty-five-minute warm ischemia is a standard model of experimental AKI, but not applicable for analysis of post-ischemic chronic renal failure. Concurrently, 90 minutes of solitary kidney warm ischemia induces exceedingly severe IRI leading to almost 100% of mortality in an acute post-ischemic phase. Thus, the model of 90-minute renal ischemia with delayed healthy kidney nephrectomy was applied as the experimental model of chronic renal failure. The surgical procedures of in situ left kidney vascular pedicle clamping followed by splenectomy and subsequent clamp release were performed under ether anesthesia. During the ischemia period rats were awaken. Twenty-four hours and 6 days after 45-minute ischemia, the animals were placed in metabolic cages for 24-hour urine collection and subsequently underwent blood sample collection from the retro-orbital plexus. Diuresis (D; mL/24 hours), creatinine clearance ($Cl_{Cr}$; mL/min), proteinuria to creatinine clearance ratio ($U_{Prot}/Cl_{Cr}$; mg/mL) and fractional excretion of sodium ($FE_{Na}$, %), as well as kidney mass (mg/100 mg body weight) were estimated 48 hours and 7 days after ischemia. In the third study, long-term renal function and mortality rate were assessed 6 months after 90-minute IRI. The study protocol was approved by the local bioethical committee for experiments on animals.

## RESULTS

As shown in Table 1, splenectomy performed simultaneously with 45-minute solitary kidney ischemia alleviated ischemia-induced kidney function impairment in a 48-hour–long observation. Seven days after IRI induction the renal function has normalized in all animals, however, significantly higher kidney mass expressed more intense inflammatory edema in the control group. Long-term observation confirmed development of post-ischemic chronic renal failure in asplenic (group 5, N = 8), as well as sham group (group 6, N = 4) reflected with increased proteinuria ($U_{Prot}/Cl_{Cr}$: 0.034 ± 0.0.02 and 0.035 ± 0.01 mg/mL, respectively); however, these results were much influenced by high mortality rate in the latter. Six-month observation revealed a twice higher survival

**Table 1. Results at 48 Hours and 7 Days After 45-minute Solitary Kidney Ischemia*,†**

| | | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|---|
| | | Splx | Sham | Splx | Sham |
| | | Upon 45-min Renal Ischemia | | 2 Weeks Before 45-min Renal Ischemia | |
| | N | 12 | 12 | 9 | 9 |
| 48 h | D | 22.08 ± 4.53 | 28.29 ± 8.72 | 27.28 ± 4.89 | 24.44 ± 8.42 |
| | $Cl_{Cr}$ | 1.32 ± 0.51 | 0.50 ± 0.33‡ | 0.74 ± 0.37 | 0.81 ± 0.30 |
| | $FE_{Na}$ | 0.76 ± 0.93 | 3.88 ± 5.11§ | 2.10 ± 1.26 | 2.31 ± 2.54 |
| | $U_{Prot}/Cl_{Cr}$ | 0.009 ± 0.007 | 0.029 ± 0.016‡ | 0.018 ± 0.015 | 0.018 ± 0.012 |
| 7 d | D | 11.79 ± 3.42 | 20.2 ± 10.14¶ | 16.56 ± 6.92 | 15.56 ± 5.75 |
| | $Cl_{Cr}$ | 3.15 ± 0.49 | 2.77 ± 0.55 | 2.81 ± 0.72 | 2.58 ± 0.67 |
| | $FE_{Na}$ | 0.07 ± 0.04 | 0.08 ± 0.05 | 0.15 ± 0.14 | 0.13 ± 0.11 |
| | $U_{Prot}/Cl_{Cr}$ | 0.001 ± 0.0004 | 0.0013 ± 0.0004 | 0.001 ± 0.001 | 0.002 ± 0.001 |
| | kidney mass | 0.63 ± 0.06 | 0.81 ± 0.25‡ | 0.67 ± 0.15 | 0.69 ± 0.1 |

Abbreviations: Splx, splenectomy; D, diuresis; $Cl_{Cr}$, creatinine clearance; $FE_{Na}$, fractional excretion of sodium; $U_{Prot}/Cl_{Cr}$, proteinuria to creatinine clearance ratio; N, number of animals.

*Splenectomy (groups 1 and 3) or sham splenectomy (groups 2 and 4) performed 2 weeks before (groups 3 and 4) or upon ischemia (groups 1 and 2).

†Means ± SD, Mann-Whitney U test: ‡ < 0.001 versus group 1; § < 0.005 versus group 1, ¶ < 0.05 versus group 1.