Background and Aim

Acute kidney injury (AKI) is an important risk factor for development of chronic kidney disease (CKD) [1], hence renal fibrosis, in humans. Renal ischemia-reperfusion injury (I/R) in rodents is a widely used model of AKI, the severity of which is dependent on body temperature during and duration of ischemia [2]. With the objective of optimizing an I/R induced model of persistent renal fibrosis in mice, we quantified the expression of 4 fibrosis-related genes, i.e. Col I, TGFβ, CCN2 (CTGF) and CCN3 (NOV), after unilateral I/R of varying severity. In addition, renal fibrosis was evaluated through quantification of Collagen I immunostain.

Materials and Methods

- Male C57BL/6J mice (n=6/condition) underwent unilateral renal ischemia/reperfusion in mice with rectal probe feedback. After reperfusion, animals were kept on a hot water pad (37°C) until awakening. Sham operated mice were included as controls.

Study setup a: effect of ischemia time

(a) To examine the effect of ischemic duration on fibrotic outcome, the left renal artery and vein were clamped for 30, 21 and 18 min at a fixed temperature of 36°C to induce ischemia. Animals were euthanized 6 or 12 weeks after I/R.

Study setup b: effect of temperature during ischemia

(b) To examine the effect of temperature during ischemia on fibrotic outcome, core body temperature was kept stable at 34°C, 35°C, 36°C and 37°C during 30 min of ischemia of the left kidney. Animals were euthanized 12 weeks after I/R.

- Serum creatinine was determined according to the Jaffe method.
- Expression of Collagen I, TGFβ, CCN2 and CCN3 was assessed by qPCR.
- Renal fibrosis was evaluated on left kidney tissue stained with Collagen I immunostain and quantified using the Axiovision image analysis software.

Results

Unilateral ischemia/reperfusion can induce persistent renal fibrosis. The extent of renal fibrosis, in terms of Col I, TGFβ, CCN2 and CCN3 gene expression, and renal collagen I deposition increases with increasing ischemic temperature and time. These data demonstrate unilateral I/R to have potential as a model to investigate and/or intervene with the long term histopathologic sequelae following an acute ischemic insult.

Conclusions

Unilateral ischemia/reperfusion can induce persistent renal fibrosis. The extent of renal fibrosis, in terms of Col I, TGFβ, CCN2 and CCN3 gene expression, and renal collagen I deposition increases with increasing ischemic temperature and time. These data demonstrate unilateral I/R to have potential as a model to investigate and/or intervene with the long term histopathologic sequelae following an acute ischemic insult.

References


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