

Use of Water Immersion to Ameliorate the Progression of Chronic Experimental Kidney Disease

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Summary

The possible benefits of aquatic environment to kidney function in renal failure stages not much been investigated. It is known that water environment could influence renal function positively: plasma renin activity is reduced, contributing to renal vascular pressure and sodium excretion. Water immersion causes increase in renal blood flow and contributes to the lowering in renal sympathetic nerve activity, renal vascular pressure and decrease in plasma renin activity. Non-swimming aerobic aquatic exercises have shown a beneficial effect to chronic kidney disease patients. We hypothesized that the aquatic environment could improve renal functioning and even slow the progression rate of chronic kidney disease (CKD). The aim of our study was to investigate the effects of regular water immersion and voluntary swimming to the rate of progression of experimental CKD. Wistar rats were divided into matched groups 2 weeks after 5/6 nephrectomy (5/6NPX) and studied during 18 weeks. One group was subjected to water immersion with water temperature 38° and swimming without exhaustion 30 min daily for 12 weeks. Control groups remained sedentary. Chronic studies of systolic blood pressure and urinary protein excretion rate (mg/24h) were performed. Renal morphology was studied and MCP-1 gene expression level was investigated in kidney tissue samples at the end of the study. The main systolic blood pressure was significantly lower and proteinuria was reduced significantly in the swimming-immersion group compared to control 5/6NPX animals. The degree of glomerulosclerosis and interstitial fibrosis was significantly less prominent in the water-therapy group. Expression of mRNA for chemokine MCP-1 in glomeruli of CKD animals differs significantly between the water-therapy group and control 5/6NPX group and was closely associated with effects on proteinuria and systolic blood pressure. These results point to the additional renoprotective properties of long-term water immersion and daily aquatic therapy in rats with CKD.

Introduction

Antihypertensive drugs are now widely in use in the therapeutic management of hypertension and chronic kidney disease. However, less known is how non-pharmacological therapies influence kidney tissue and whether these could give additional renoprotective

properties. Exercise therapy is always related to stress and used for chronic kidney patients with care (*Painter, 2005*). Blood flow during exercise is preferentially directed to working muscle, and away from other central organs like the kidney (*Clorius et al., 1996*). Distribution of blood flow is influenced by sympathetic arterial vasoconstriction and is pronounced in hypertensive individuals (*Clorius et al., 2002*). Sympathetic over-activity is triggered by kidneys, contributing to hypertension (*Rump et al., 2000*) and nephrosclerosis (*Amann et al., 2000*). Nevertheless, it has been shown, both in experimental and clinical studies, that well designed long-term

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aerobic exercise could decrease blood pressure, lower the sympathetic over-activity, improve lipid profile, diminish proteinuria, protect kidney tissue from oxidative stress (Kohzuki *et al.*, 2001; Pechter *et al.*, 2003; Ravi Kiran *et al.*, 2004; Goto *et al.*, 2007). Also that exercise has an impact on inflammatory mediators in the cardiac tissue of CFR rats (Bai *et al.*, 2008).

Mostly used exercise in the animal studies is forced treadmill running or swimming to exhaustion (Kanzawa *et al.*, 2006). Water immersion causes increase in renal blood flow and contributes to the lowering in renal sympathetic nerve activity, renal vascular pressure and decrease in plasma renin activity (Epstein, 1992; Becker *et al.*, 1998). Some experimental studies have shown that exercise in a water environment could improve renal functioning and even slow the disease progression rate (Heifets *et al.*, 1987; Osato *et al.*, 1990). Aquatic immersion creates physiologically non-invasive effects on renal regulatory systems, as has been published by Epstein (1992): renal blood flow increases upon immersion. Water immersion *per se* abates the renal vasoconstriction during exercise. Thus, renal vasoconstrictive risks of land-based exercise can be avoided when exercising in an aquatic environment. Our study was designed to explore the rate of progression of renal disease in an experimental model of chronic renal failure and hypertension (remnant kidney) after 5/6 nephrectomy in Wistar male rats measured by physiological parameters and investigated in kidney tissue samples after 12 weeks of regular water therapy consisting of non-exhaustive water immersion and voluntary exercise.

Materials and Methods

The Animal Studies Ethics Committee of the Tartu University approved the study protocol. Male Wistar rats were purchased from the Laboratory Animal Center, University of Kuopio, Finland. An acclimatization period of 10 days was allowed before any experiment work was undertaken. Rats were kept in a climate-controlled facility at the Faculty of Medicine of the University of Tartu under standard

conditions on a 12-h light/dark cycle and fed with standard rodent chow (R 70, Lactamin AB, Sweden) and tap water ad libitum.

Experimental Design

The rats were subjected to subtotal (5/6) nephrectomy (NPX) as previously described (Ots *et al.*, 1998) at week (wk) 0. At approximately 10 wks of age, rats weighing 360-400 g were anesthetized with intraperitoneal methohexital sodium, 5 mg per 100 g body weight. Renal ablation was then accomplished by right nephrectomy and selective ligation of extrarenal branches of the left renal artery in such a way that approximately 2/3 of the left kidney was infarcted. Two weeks later 25 rats were matched for age and body weight and divided into four groups and studied during 12 weeks of therapy: Group I (n=10) - aquatic therapy for NPX animals. Group II (n=5) - healthy control. Group III (n=4) - sham-operated healthy control. Group IV, NPX control group (n=6) - NPX animals without treatment. Body weight was measured biweekly for the duration of the study. Systolic blood pressure (SBP, mmHg) was measured biweekly by the tail-cuff manometer (Harvard Apparatus, USA) in awake pre-warmed rats. The urine was collected for 24 hours (h) using metabolic cages, for determination of proteinuria (Uprot, g/24h) that was measured with a Hitachi 912 Analyser.

Water-therapy

10 animals (aquatic therapy, group I) were subjected to thermoneutral (water temperature 38°) immersion and voluntary swimming without exhaustion 30 min daily for 12 weeks in a water pool with water depth of 50 cm (Figure1). Control groups remained sedentary.

Morphological studies

At the end of the study, rats were anesthetized using methohexital sodium (50 mg per 100g bodyweight). The remnant kidneys were removed and tissue fixed in 10% buffered formaldehyde. Paraffin sections of coronal slices, through the pelvis of the remnant



Figure 1. Water immersion and voluntary swimming without exhaustion 30 min daily for 12 weeks in water pool with water depth of 50 cm.

kidney, were cut at 4 mm thickness and stained using the periodic acid-Schiff (PAS) and Masson's trichrome methods. Periodic acid-Schiff sections from each kidney were studied morphologically for evidence of focal-segmental glomerulosclerosis (FSGS), defined as glomeruli showing evidence of segmental or global collapse of capillaries with or without associated hyaline deposition and adhesions of the capillary tuft to Bowman's capsule. The extent of FSGS was expressed as a percentage of the total number of glomeruli counted (>50/section). Trichrome stained sections from each kidney were graded for the presence of interstitial fibrosis (0, 1+, 2+, 3+).

Quantification of rat MCP-1 mRNA by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR):

For quantification of rat *MCP-1*, or alternatively *Ccl2* (monocyte chemoattractant protein-1 in rat), and endogenous reference *β-actin* mRNA, we used a SYBR Green real-time quantitative RT-PCR method based on the TaqMan fluorescence method with the ABI Prism 7000 Sequence Detection System (PE Applied Systems, Foster City, California). Total RNA was extracted from kidney tissue sam-

ples by using Rneasy Mini Kit (Qiagen) and cDNA synthesized with a First-Strand cDNA Synthesis Kit SuperScript™ III (Invitrogen). RNA was quantified by determination of ultraviolet absorbance at 260 nm, and purity was assessed by measuring the optical density ratio at 260 and 280 nm (Nanodrop). Transcripts levels for rat *MCP-1* (*Ccl2*) and *β-actin* were quantified using SYBR Green-based quantitative real-time PCR technology. Amplification was performed by using the SuperArray's (nowadays SABiosciences) RT SYBR Green/ROX qPCR Master Mix, other reagents and Real-Time RT² qPCR Primer Assays. The polymerase chain reaction was performed as described by the manufacturers (www.SABiosciences.com). The RT² qPCR oligonucleotide primer sets for rat *MCP-1* and *β-actin* from SABiosciences were used to produce the gene-specific PCR amplicon products of the correct size. For amplification quality control, we performed the dissociation curve program immediately after the PCR program and carried out the agarose gel electrophoresis (Figure 2). Amplification efficiencies of endogenous reference and target sequence were comparable, if we prepared the standard curves in an initial experiment. The mRNA levels of the target sequence were normalized to those of *β-actin* as a housekeeping gene, and used as an endogenous internal control; the relative levels of each mRNA to that of *β-actin* were calculated. PCR reactions for both factors were repeated in triplicate. XpressRef™ Universal Total RNA product from SABiosciences was used as the interassay standard.

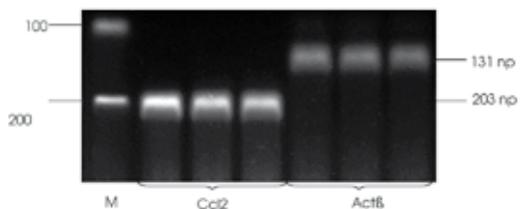


Figure 2. An example of RT-PCR quality control. Agaros gel (4,5%) electrophoresis of *Ccl2* *Actβ* amplicons 203 np and 131 np, respectively.

Statistical analysis

Data were collected at baseline and after 12 weeks of water therapy and are presented as mean values ± SD. Data were analyzed by one-time ANOVA with the Tukey-Kramer test for comparisons significant at the 0,05 level or repeated ANOVA measures with post-hoc testing as appropriate using the commercially available statistical package SAS.

Statistical analysis for RT-qPCR: The relative amount of MCP-1 (*Ccl2*) expression in the rat kidney cortex probes was calculated using Delta-Delta Ct method. The difference in expression level is given by $2^{-\Delta\Delta Ct}$. Differences between groups were examined for statistical significance using Student *t*-test and Mann – Whitney rank test. P< 0.05 was considered statistically significant.

Correlations were assessed using a one-sample *t* test for a correlation coefficient. The null hypothesis was rejected at p < 0.05.

Results

There were no significant differences in body weight among the studied groups. Average levels of systolic blood pressure (SBP) in the water treatment group were significantly lower than in untreated animals (p<0.05). Proteinuria (UprotV) was significantly reduced in the water-therapy group compared with untreated animals (p<0.05). There were significant differences in healthy control and sham-operated animals in comparison with NPX animals (Table 1).

Table 1. Results. Systolic blood pressure (SBP) and proteinuria after 12 weeks of study period.

Group	SBP (mmHg)	Proteinuria (g/24h)
Group I – 5/6NPX with water-therapy	127,92 ± 5,88 *	2,13 ± 0,42 *
Group II – healthy control	101,50 ± 4,70 *	0,02 ± 0,00 *
Group III - sham-op	103,50 ± 4,55 *	0,52 ± 0,19 *
Group IV –5/6PX without water-therapy	150,33 ± 2,96	5,56 ± 1,58

* p < 0,05 vs. group IV

Less glomerulosclerosis (FSGS) was found in remnant kidneys of the water-therapy group and the interstitial fibrosis (IF) score was lower compared with untreated NPX-group controls. No morphological changes were found in healthy control and sham-operated animal groups. The difference between untreated groups and healthy controls was significant (p<0.05). Indices measured in the kidney tissue are presented in Table 2.

Table 2. Results. Focal-segmental glomerulosclerosis (FSGS) and interstitial fibrosis (IF) after 12 weeks of study period.

Group	FSGS (%)	IF (0..3)
Group I – 5/6NPX with water-therapy	18.10 ± 2.17*	0,55 ± 0,42*
Group II – healthy control	0,00 ± 0,00*	0,00 ± 0,00*
Group III - sham-operated	0,00 ± 0,00*	0,00 ± 0,00*
Group IV –5/6NPX without water-therapy	31.17 ± 2.87	1,33 ± 0,48

* p < 0,05 vs. group IV

RT qPCR analysis revealed that the differences of mRNA expression for MCP-1 between water therapy group (I) and sedentary control group (IV) were statistically significant (p<0.002). The expression of MCP-1 mRNA was lower in the water-therapy group (I) than in the sedentary control group (IV). Data were expressed as ratios of the amount of expressed mRNA of factor MCP-1 to that of β-actin. MCP-1 values in kidney tissue were found to be correlated with 24h proteinuria among the individual rats pooled from water therapy group through NPX (p = 0.02, r = 0.59).

Discussion

After reduction in renal mass in the NPX model, the remaining nephrons undergo functional as well as structural hypertrophy; glomerular and systemic hypertension develops. The lesions which develop in remnant glomeruli of rats after subtotal nephrectomy resemble those seen in a variety of human chronic renal diseases. This remnant kidney model used in our study enables one to evaluate the dy-

namics of physiological parameters in comparison to local changes in kidney tissue (renal pathomorphology) in chronic renal failure (*Ots 1998*). Previous experimental studies have shown that moderate exercising does not worsen renal function; moreover, it helps to attenuate the increase in proteinuria and blood pressure (*Heifets 1987; Adams et al., 2005*). However, it is necessary to investigate various beneficial tactics that could protect kidney tissue from progressive damage. This animal model of progressive kidney disease gives the possibility to evaluate the safety and possible benefits of different treatments, including non-pharmacological, in the care of chronic renal patients. Protective therapy may have the greatest impact if initiated early in the course of renal failure development.

The results of our study show that water immersion with voluntary exercise repeated chronically as a treatment in a long time period (12 weeks) gives renoprotective effects in this model of renal insufficiency.

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