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INTRAVENOUSLY ADMINISTERED SIRNA ACCUMULATES IN THE KIDNEY AND SELECTIVELY SUPPRESSES GENE FUNCTION IN RENAL PROXIMAL TUBULES

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Abstract

Different gene-silencing methods, like antisense and short interfering RNA (siRNA), are widely used as experimental tools to inhibit gene expression. In the present study, the *in vivo* behavior of siRNA in rats and siRNA-mediated silencing of genes in the renal proximal tubule were investigated. To study the biodistribution of siRNA, rats were injected intravenously with radiolabeled-siRNA or radiolabel alone (control) and scintigraphic images were acquired at different time-intervals post-injection. SiRNA preferentially accumulated in the kidneys and was excreted in the urine. One hour after injection the amount of siRNA present in both kidneys (1.7 ± 0.3 % of injected dose/g tissue) was on average 40 times higher than in other tissues (liver, brain, intestine, muscle, lung, spleen, blood). Besides the biodistribution, the effect of siRNA on multidrug resistance protein isoform 2 (Mrp2/Abcc2; $siRNA_{Mrp2}$) in renal proximal tubules was investigated. Mrp2 function was assessed by measuring the excretion of its fluorescent substrate calcein in the isolated perfused rat kidney. Four days after administration, $siRNA_{Mrp2}$ reduced the urinary calcein excretion rate significantly (35 % inhibition over the period 80-150 min of perfusion). This down-regulation was specific, since another siRNA sequence directed against a different transporter in the proximal tubule, Mrp4 (Abcc4; $siRNA_{Mrp4}$), did not alter the Mrp2 mediated excretion of calcein. In conclusion, siRNA accumulates spontaneously in the kidney after intravenous injection, where it selectively suppresses gene function in the proximal tubules. Therefore, intravenously administered siRNA provides a novel experimental and potential therapeutic tool for gene silencing in the kidney.

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Various gene silencing methods, like antisense oligonucleotides and short interfering RNA (siRNA), are widely used as experimental tools to inhibit the expression of genes. During the last few decades, antisense oligonucleotides have been used to study gene function by silencing gene expression *in vitro* and *in vivo*. In addition, several antisense oligonucleotides have entered into clinical trials and in 1998, the first antisense drug, Fomivirsen, was approved by the US Food and Drug Administration (Marwick, 1998). Because of the potential application of antisense oligonucleotides as therapeutic agents, several research groups investigated the biodistribution and pharmacokinetics of antisense oligonucleotides (reviewed by Levin (1999)). After intravenous administration, antisense oligonucleotides are rapidly cleared from the plasma and distributed to the peripheral tissues. In rats, phosphorothioate antisense oligonucleotides are mainly taken up by the kidney (Bijsterbosch et al., 1997; Lendvai et al., 2005). Studies on the intra-renal distribution of antisense oligonucleotides showed that oligonucleotide uptake is most prominent in the proximal tubule (Carome et al., 1997; Oberbauer et al., 1995). This specific uptake takes place via filtration by the glomeruli followed by tubular reabsorption, as well as by direct uptake at the capillary side of the tubules (Sawai et al., 1996).

SiRNA was discovered more recently as a promising gene silencing tool in research and in the clinic. The molecular mechanism of RNA interference (RNAi) was first described by Fire *et al*. (1998), who observed a sequence specific gene silencing at the post-transcriptional level in the nematode *Caenorhabditis elegans* after exposure to double stranded RNA (dsRNA). In the RNAi pathway, long dsRNA sequences are cleaved by the enzyme dicer into short 21-base pair stretches of dsRNA, the short interfering RNAs. These siRNA duplexes associate with a multi-protein complex known as the RNA-induced silencing complex (RISC). After unwinding, siRNA strands bind to complementary mRNA, causing a targeted degradation of mRNA and, thus, a translational block. The finding that exposure to siRNA duplexes shorter

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than 30 base pairs circumvent activation of the interferon pathway and overall suppression of gene expression enabled the successful use of this technique in mammalian cells (Elbashir et al., 2001; Tuschl and Borkhardt, 2002). Compared to antisense oligonucleotides, siRNA duplexes seem to be more resistant to biodegradation in cell culture and more efficient in silencing gene expression (Bertrand et al., 2002; Grunweller et al., 2003).

Besides the applications of siRNA *in vitro*, a great interest exists to apply this technique as an experimental or therapeutic tool *in vivo*. To achieve this, different experimental methods have been used to enhance effective delivery of siRNA to the target organs. For example, hydrodynamic injection of siRNAs and cationic liposome-mediated delivery of siRNAs in mice were effective in silencing gene expression in different organs *in vivo* (Lewis et al., 2002; Sioud and Sorensen, 2003; Song et al., 2003; Sorensen et al., 2003). However, despite the successful silencing of genes by siRNA *in vivo*, the effective delivery of siRNA to the target organs still forms a major obstacle and little is known about the *in vivo* behavior of synthetic siRNA duplexes.

The objective of the present study was to investigate whether siRNA duplexes, like antisense oligonucleotides, are delivered spontaneously to the kidney and whether siRNA can silence genes *in vivo*. Our results showed that siRNA accumulates rapidly in rat kidneys after intravenous injection. In addition, we showed effective silencing of a transporter gene in the kidney proximal tubule.

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Methods

SiRNA sequences and preparation

In order to investigate the *in vivo* behavior of siRNA, the biodistribution of a duplex RNA containing two phosphodiester (PO/PO) RNA strands and a dTdT overhang was studied. The siRNA sequence was directed against rat multidrug resistance protein 4 (Mrp4; $siRNA_{Mrp4}$), and contained a 3'-amino C7 linker on the antisense strand for radio-active labeling of the siRNA duplex. To measure the functional effects of siRNA, the same $siRNA_{Mrp4}$ sequence without 3'-amino C7 linker and a siRNA sequence against rat Mrp2 ($siRNA_{Mrp2}$) were used. The DNA target sequences were 5'-AACATGGCCTACTCCTACCTG-3' for $siRNA_{Mrp2}$ and 5'-AAGAGAGGTGTCAGGAGCTGT-3' for $siRNA_{Mrp4}$. To avoid formation of higher aggregates, siRNA duplexes were prepared by dissolving the duplexes in siRNA suspension buffer, followed by heating at 90 °C for 1 min and incubation at 37 °C for 1 hour, according to the manufacturers' instructions (Qiagen, Venlo, The Netherlands).

Radiolabeling of siRNA

Duplex 3'-amino $siRNA_{Mrp4}$ was conjugated with cDTPA (Sigma, St. Louis, MO, USA) and subsequently labeled with Indium-111 (^{111}In; half-life 2.8 days) under strict RNase-free conditions. All solutions were supplemented with 0.2% (v/v) diethylpyrocarbonate (Fluka, Buchs, Switzerland). All solutions and pipet tips were autoclaved. SiRNA (20 nmol) was solubilized in 10 µl suspension buffer and prepared as described above. The pH of the solution was adjusted to pH 8.2 by adding 10 µl 0.2 M $NaHCO_3$. A 50-fold molar excess of cDTPA solubilized in 36 µl dimethylsulfoxide was added to the siRNA solution and the reaction mixture was incubated 45 min at room temperature. The reaction was stopped by adding 180 µl 0.1 M citrate buffer, pH 5.0 and the fraction mixture was dialyzed extensively

against 0.1 M citrate buffer, pH 5.0 in a Slide-A-Lyzer with a molecular cut-off of 3,000 Da (Pierce, Rockford, IL, USA). The siRNA-DTPA conjugate was stored in aliquots at -20 °C until use.

For radiolabeling, we used 10 nmol siRNA-DTPA conjugate in 100 μl in 0.1 M citrate buffer, pH 5.0. 500 μCi $^{111}InCl_3$ (Tyco Medical, Petten, The Netherlands) was added and the mixture was incubated during 30 min at room temperature. The ^{111}In-labeled DTPA-siRNA was purified by gel filtration on a disposable G25M sephadex column (PD10, Pharmacia, Woerden, The Netherlands) eluted with phosphate buffered saline. The specific activity of the final preparation was 30 μCi/nmol. The radiochemical purity of the preparation was tested by eluting a sample of the preparation on a PD10 column and exceeded 95%.

In vivo experiments

The Animal Experimental Committee of the Radboud University Nijmegen approved all procedures involving animals. SPF-bred Male Wistar Hannover (WH) rats (10-12 weeks) were obtained from Harlan (CPB, Zeist, The Netherlands). WH rats and Mrp2-deficient TR$^-$ rats (de Vries et al., 1989) were housed under routine laboratory conditions at the Central Animal Facility Nijmegen.

Biodistribution of radiolabeled siRNA

To study the biodistribution of ^{111}In-labeled DTPA-siRNA three rats were injected intravenously with 50 μCi ^{111}In-labeled DTPA-siRNA diluted in 200 μl PBS. A control group of three rats was injected intravenously with 50 μCi ^{111}In-labeled DTPA. The rats were placed on a gamma camera (Siemens Orbitor, Hoffmann Estates, IL, USA) equipped with a medium energy collimator and scintigraphic images were acquired at 5, 30 and 60 min post-injection. Rats were euthanized at 1 hour post-injection and the biodistribution of the radiolabel was

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determined. A blood sample was drawn by cardiac puncture and normal tissues (muscle, lung, spleen, kidney, liver, small intestines and brain) were dissected, weighed, and counted in a gamma counter (Wizard; Pharmacia-LKB, Uppsala, Sweden). To correct for radioactive decay, injection standards were counted simultaneously. The activity in samples was expressed as percentage of the injected dose per gram tissue (%ID/g).

Functional assessment of siRNA treatment

To investigate the effects of $siRNA_{Mrp2}$ and $siRNA_{Mrp4}$ on Mrp2 transport function, male WH rats were injected in the tail vein with 7 nmol (100 μg) $siRNA_{Mrp2}$ or $siRNA_{Mrp4}$ dissolved in 150 μl siRNA suspension buffer. Dependent on the experimental settings, rats were euthanized 3 or 4 days post-injection. Rat kidneys were isolated and perfused with calcein-acetoxymethylester (calcein-AM), a lipophilic compound which is converted into calcein intracellulary. Subsequently, the urinary excretion of calcein, a fluorescent substrate of Mrp2, was determined. The isolation of rat kidneys and *in vitro* perfusion were performed as described previously in detail (Masereeuw et al., 2003). TR^- rats, lacking Mrp2 expression, were used to compare siRNA effects on Mrp2 transport function.

Data analysis

Data were analyzed using GraphPad Prism version 4.02 for windows (GraphPad software, San Diego CA, USA). Data are given as mean ± sem. Mean values were considered to be significantly different, when $p<0.05$ by use of a student's *t*-test, one-way ANOVA or by a two-way ANOVA both followed by Bonferroni's multiple comparison test.

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Results

Biodistribution of siRNA

The *in vivo* distribution of siRNA was studied in rats after intravenous injection of radiolabeled siRNA. As a control, rats were injected with the radiolabel alone. Figure 1 shows the scintigraphic images of rats acquired after intravenous injection with radiolabeled siRNA or radiolabel. After an initial rapid distribution phase, radioactivity is cleared from the circulation via the kidneys and excreted into the urine. Compared with control (^{111}In-DTPA), higher concentrations of ^{111}In-DTPA-siRNA were found in both kidneys, indicating that the kidney selectively takes up siRNA after intravenous administration. In figure 1, one kidney of a rat injected with ^{111}In-DTPA-siRNA shows a very high accumulation of siRNA compared with the other kidneys. This kidney was not included in the biodistribution shown in figure 2.

Rats were euthanized 1 hour after intravenous injection and the radioactivity per organ was determined by calculating the percentage of injected dose per gram tissue (%ID/g) recovered in each organ. Figure 2 shows that a relatively high concentration of radiolabeled siRNA is found in the kidneys. Here 1.7 ± 0.3 % of the injected dose per gram tissue is present 1 hour after injection. Accumulation of siRNA in the kidneys is almost three times as high as radiolabel alone (0.6 ± 0.1 %ID/g). One hour after injection, the radioactivity present in the circulation is somewhat lower in siRNA treated rats (0.07 ± 0.01 %ID/g) as compared with control rats (0.10 ± 0.01 %ID/g). There is no selective uptake of siRNA in the muscles, lungs, spleen, liver, intestines and brain. In support, the concentration of radiolabel in these organs is very low (<0.07 %ID/g).

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Functional assessment of siRNA treatment

The effects of siRNA duplexes were tested through assessment of the multidrug resistance associated protein isoform 2 (Abcc2/Mrp2). As a control, siRNA against rat Mrp4 (Abcc4) was used. MRP2 and MRP4 are members of the MRP family (MRP1-9), belonging to the subfamily C of the ATP-binding cassette (ABC) transporters. These two transport proteins are expressed in the brush-border membrane of the kidney proximal tubule (Schaub et al., 1997; van Aubel et al., 2002), where they excrete a broad range of organic anionic compounds into the urine (van de Water et al., 2005). We showed previously that Mrp2 function can be monitored using an isolated perfused rat kidney model and calcein as a substrate (Masereeuw et al., 2003). Isolated kidneys were perfused with medium containing calcein-AM, which was converted intracellularly by esterases into the fluorescent Mrp2 substrate calcein. In Mrp2-deficient (TR^-) rats, the urinary calcein excretion was found to be highly impaired due to the lack of a functional Mrp2 (Masereeuw et al., 2003).

Four days after exposure of rats to a single dose of $siRNA_{Mrp2}$, a significant inhibition of the calcein excretion was found as compared to normal WH rats, indicating a reduced Mrp2 function (Figure 3). With the excretion in Mrp2-deficient TR^- rats set as a baseline, a 35% inhibition of Mrp2 function was observed in $siRNA_{Mrp2}$ treated rats over the period 80-150 min of perfusion. Inhibition of calcein excretion was already observed three days post-injection ($p<0.001$ at $t = 60$-70 min, data not shown), but the functional knock-down of Mrp2 was highest four days after injection. Injection of rats with $siRNA_{Mrp4}$ did not significantly reduce Mrp2 function. This shows that other siRNA sequences, which are not complementary to Mrp2 mRNA, do not result in a non-specific silencing of Mrp2.

Besides the effects of siRNA on Mrp2 transport function, general functional parameters of the kidney were monitored during the perfusion experiment. Table 1 presents the mean glomerular filtration rate, diuresis, fractional reabsorption of water and perfusion pressure for

the different experimental groups. Administration of both siRNA sequences did not affect these parameters. In contrast, glomerular filtration rate and diuresis were increased in TR^- rats compared with controls ($p<0.01$). This is most probably the result of a somewhat higher renal perfusion pressure during the experiments. Nevertheless, renal function of TR^- rats was good during the experimental time period, which is in agreement with our previous findings for these rats (Masereeuw et al., 2003).

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Discussion

A new promising tool to silence gene expression *in vivo* is siRNA, but little is known about the distribution of siRNA duplexes. Thus far, successful delivery of siRNA in mammals *in vivo* was investigated in mice using techniques like hydrodynamic injection via the tail vein. However, intravenous injection of siRNA under these conditions in humans is not feasible. Therefore, the analysis of the biodistribution of siRNA and exploration of other delivery methods may contribute to a more effective use of siRNA.

The present study clearly demonstrates that after intravenous administration of even small amounts, siRNA preferentially accumulates in the kidney. After tail-vein injection, the sequences rapidly distributed throughout the body and a large amount was delivered to the kidneys and excreted into the urine. One hour after injection, the amount of siRNA present in the kidneys was about 40 times higher than in the other organs. Although the biodistribution was studied with an siRNA sequence against Mrp4, we expect to find a similar biodistribution pattern with siRNA duplexes against any other gene. The functional down-regulation of Mrp2 in the kidney is in support of this.

Braasch *et al*. (2004) showed previously the biodistribution of siRNA in mice at 0-72 hours after intravenous injection. In contrast to our findings, they found that ^{125}I-labeled siRNA accumulated in the liver in addition to the kidney. This difference may be caused by the siRNA sequence and the radiolabeling method used, and/or by interspecies differences. Due to its hydrophilic character, the DTPA-linker we used for radioactive labeling of siRNA is easily filtered by the glomerulus, after which the complexes are specifically taken up by the renal proximal tubules. In accordance with Braasch *et al*., we detected very low siRNA concentrations in the brain, most likely reflecting a poor ability of siRNA to penetrate the blood-brain barrier.

The siRNA biodistribution pattern is similar to that found after intravenous injection of antisense oligonucleotides (Bijsterbosch et al., 1997; Lendvai et al., 2005), which is most likely independent of the sequence studied (Rifai et al., 1996).

For siRNA duplexes, the intrarenal distribution has not been investigated yet. However, due to the high reabsorption capacity of the proximal tubule, duplexes may, like antisense oligonucleotides, accumulate in this part of the nephron. Previous studies showed that antisense oligonucleotides predominantly accumulate in the proximal tubule cells after intravenous administration (Carome et al., 1997; Oberbauer et al., 1995). Uptake in these cells takes place via the capillary side as well as via reabsorption of antisense oligonucleotides from the tubular lumen. Compared with the capillary side, uptake at the lumenal side is much more efficient (Sawai et al., 1996). The uptake at both sites is saturable, indicating the involvement of receptor-mediated processes (Rappaport et al., 1995; Sawai et al., 1996). Although the receptors responsible for the uptake at both sites of the proximal tubule have not been identified yet, different groups showed the presence of oligonucleotide receptor proteins (Bijsterbosch et al., 1997; Rappaport et al., 1995; Sawai et al., 1996). In addition, two receptors expressed on the brush-border membrane of proximal tubule cells, cubulin and megalin, may play a role in the reabsorption of oligonucleotides from the glomerular filtrate (Birn et al., 2000; Sawai et al., 1996; Zhai et al., 2000).

Besides the application of synthetic siRNA duplexes of 21 nucleotides, RNA silencing can be induced by siRNA expressing viral and non-viral vectors. Due to their different physical properties, biodistribution patterns of these vectors may be different from those of short 21-nucleotide siRNA duplexes. With a few successful exceptions, the kidney has been quite difficult to transduce with the various vectors available (Favre et al., 2000). DNA-vectors can transduce proximal tubular cells by filtration through the glomerulus and reabsorption by the proximal tubule. DNA-complexes that are unable to pass the glomerular basement membrane

are most likely not effective to transduce proximal tubular cells via the basolateral site either (Favre et al., 2000; Foglieni et al., 2000).

A research area in which siRNA can provide a useful tool in the characterization of gene function and the development of new therapies is that of drug transport proteins. Mrp2 and P-glycoprotein (MDR1; ABCB1), for example, play an important role in the excretion of many compounds and multidrug resistance in cancer is associated with high expression levels of these transporters. SiRNA can be useful in the identification of new substrates of these transporters as well as in therapy. An excellent overview about the application of siRNA in the study of drug transport proteins has been published recently by Tian *et al*. (2005).

The present study demonstrates that after intravenous administration, siRNA accumulation in the kidney was sufficient to knock-down transporter gene function. Intravenous administration of $siRNA_{Mrp2}$ resulted in a specific inhibition of Mrp2 function. We used an isolated rat kidney model perfused with calcein-AM to study Mrp2 function. This lipophilic compound diffuses into the cells, where it is converted by esterases into calcein. This fluorescent compound is excreted into urine by Mrp2 and not by Mrp4, and can be used as a model substrate to monitor Mrp2 function. We showed previously that calcein is an excellent model substrate for monitoring Mrp2 function in the isolated perfused rat kidney (Masereeuw et al., 2003), but we cannot exclude completely the involvement of other Mrp's, *viz.* Mrp1, 3, 5 and 6, in calcein excretion. In contrast to Mrp2 and 4, Mrp1, 3, 5 and 6 are localized in the basolateral membrane, of which only Mrp6 is expressed in the proximal tubule. Therefore, changes in Mrp1, 3 and 5 expression may not have influenced the urinary calcein excretion. Mrp6 might contribute to overall renal calcein handling, however, the substrate specificity of Mrp6 is very narrow. So far, calcein has not been identified as an Mrp6 substrate. In addition, exposure to $siRNA_{Mrp4}$ did not affect calcein excretion, suggesting that there were no aspecific effects on other Mrp transporters.

The effects of siRNA on protein expression largely depend on siRNA concentrations and stability, as well as the turnover rate of the protein of interest. Compared with antisense oligonucleotides, siRNA duplexes are relatively stable and degradation of siRNA in serum is slow (Bertrand et al., 2002; Braasch et al., 2004). Expression of Mrp2 in the apical membrane of the proximal tubule is dependent on *de novo* protein synthesis, insertion into the apical membrane and retrieval from the membrane followed by degradation. Jones *et al.* (2005) investigated the rate of Mrp2 protein synthesis and degradation in rat liver. Using *in vivo* metabolic labeling and autoradiography, they estimated the degradation half-life of Mrp2 on 27 hours. With a half-life of 27 hours for Mrp2, siRNA silencing effects on this protein can be detectable within several days after siRNA administration. Using the isolated perfused rat kidney model, we observed a clear inhibition of Mrp2 function on day 3 and day 4 after injection. Unfortunately, we did not detect an inhibition of transporter expression after administration of siRNA using real-time RT-PCR or semi-quantitative Western blotting (data not shown). Although mRNA and protein expression must be down-regulated as well, in our hands these two methods were not sensitive enough to significantly detect small changes in expression levels.

To test whether siRNA administration does not alter kidney function, different parameters were analyzed during the perfusion experiments. In siRNA treated rats basic renal functional parameters, like glomerular filtration rate, diuresis, fractional reabsorption of water and renal perfusion pressure, were not different from control values, indicating that siRNA administration is safe and without side effects.

The use of siRNA has an advantage over the use of chemical inhibitors to reduce Mrp2 transport function, because it shows a specific down-regulation. To date no inhibitors have been identified that exclusively affect Mrp2. In addition, chemical inhibitors may have side effects. The spontaneous accumulation of siRNA duplexes in the kidney after intravenous

administration has potential therapeutic implications. Intravenous administration of siRNA duplexes seems a simple, but effective method in research and therapy to silence gene expression in the kidney. A more site-directed administration, like injection of siRNA into the renal artery, may even further enhance the accumulation of siRNA duplexes in the kidney.

In summary, the present study shows that after intravenous injection in rats, siRNA duplexes are distributed throughout the body within minutes. Most of the intravenously administered siRNA accumulates rapidly in the kidneys and is excreted via the urine during time. Moreover, we show for the first time successful suppression of Mrp2 in the renal proximal tubule. Therefore, intravenous administration of siRNA duplexes provides a novel research and potential therapeutic tool for gene silencing in the kidney.

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Footnotes

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Legends for figures

Figure 1:

Scintigraphic images of the uptake of radiolabeled siRNA. Rats (n=3 for each treatment group) were injected intravenously with either ^{111}In-DTPA (control) or ^{111}In-DTPA-siRNA$_{Mrp4}$ and the biodistribution of radiolabel was followed on a gamma-camera for 1 hour.

Figure 2:

Biodistribution of radiolabeled siRNA. Rats were injected intravenously with either ^{111}In-DTPA (control) or ^{111}In-DTPA-siRNA (siRNA). One hour after injection, rats were sacrificed and the distribution of the radiolabel was determined in different tissues. Radioactivity for each organ was calculated as percentage of injected dose per gram (%ID/g). Data are expressed as mean ± sem of three animals per treatment group, except for the kidneys (n=5 for siRNA treated rats and n=6 for control rats). Statistical comparisons between control and siRNA group were performed by student's *t*-tests. Significantly different from controls, *$p<0.05$, **$p<0.0001$.

Figure 3:

Renal excretion rate of calcein as a function of time in isolated perfused rat kidneys. A concentration of 100 nM of the hydrophobic, non-fluorescent, compound calcein-AM was added to normal perfusion medium and secretion of the fluorescent calcein into urine was measured in WH rats which were untreated (●, n=16), treated for four days with siRNA$_{Mrp2}$ (■, n=5), treated with siRNA$_{Mrp4}$ (▲, n=6) or in TR$^-$ rats (▼, n=4). Data are given as mean ± sem. Statistical comparisons were performed by two-way ANOVA. * Significantly different from untreated WH rats ($p<0.001$).

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Table 1. Functional parameters of isolated perfused kidneys of control rats (no additional treatment), after four days of treatment with $siRNA_{Mrp2}$, $siRNA_{Mrp4}$ and in TR^- rats [a]

Parameter	control (n=16)	$siRNA_{Mrp2}$ (n=4)	$siRNA_{Mrp4}$ (n=6)	TR^- (n=4)
GFR (µl/min)	280 ± 20	270 ± 30	240 ± 20	430* ± 40
diuresis (µl/min)	18 ± 1	16 ± 1	16 ± 1	28* ± 3
FR_{water} (%)	93.7 ± 0.2	94.0 ± 0.5	93.2 ± 0.6	93.5 ± 0.2
RPP (mmHg)	82 ± 1	81 ± 1	80 ± 2	95* ± 5

[a]Values are presented as mean ± sem over the period 30-120 min. Kidneys were perfused for 150 min as described in **Methods**. GFR = glomerular filtration rate, FR = fractional reabsorption, RPP = renal perfusion pressure. Statistical comparisons were performed by one-way ANOVA. * Significantly different from control rat kidney ($p<0.01$).

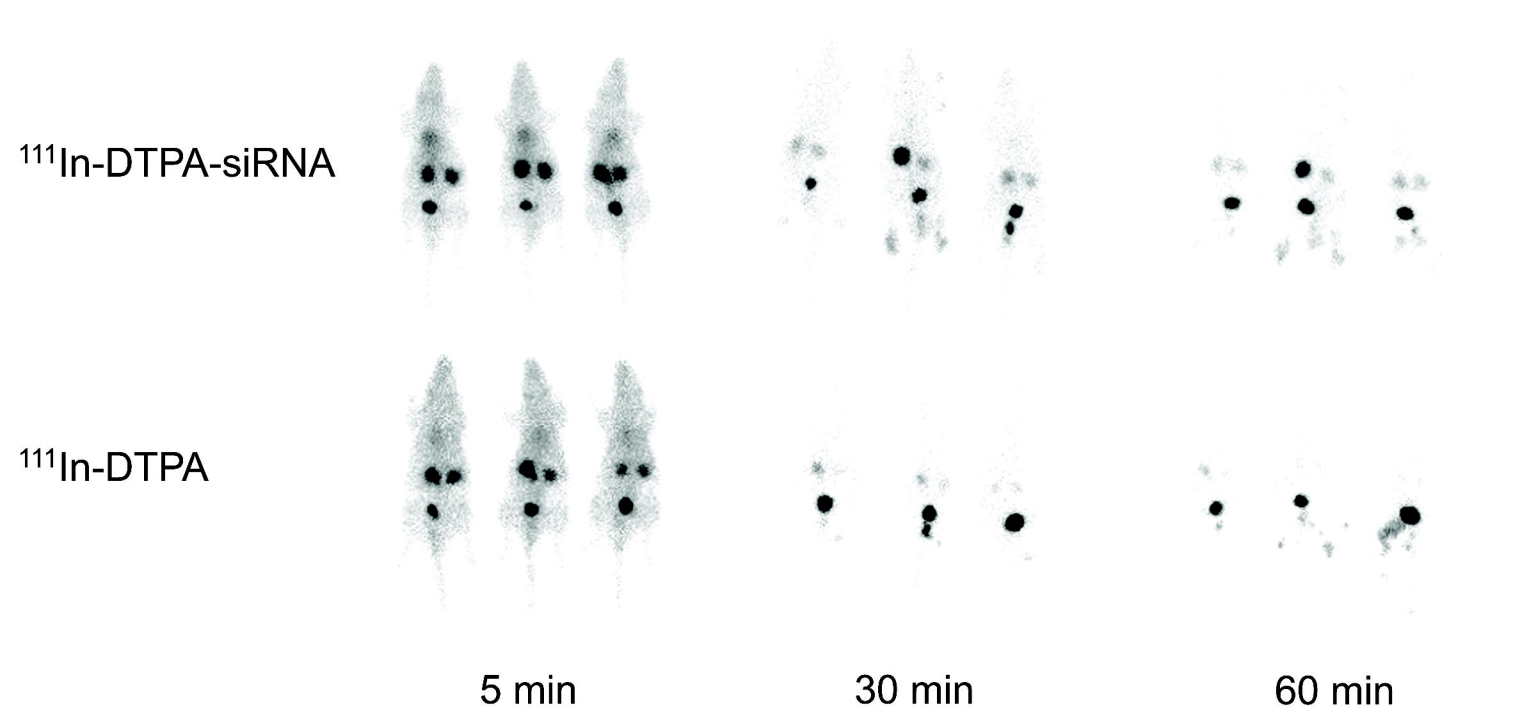

Figure 1

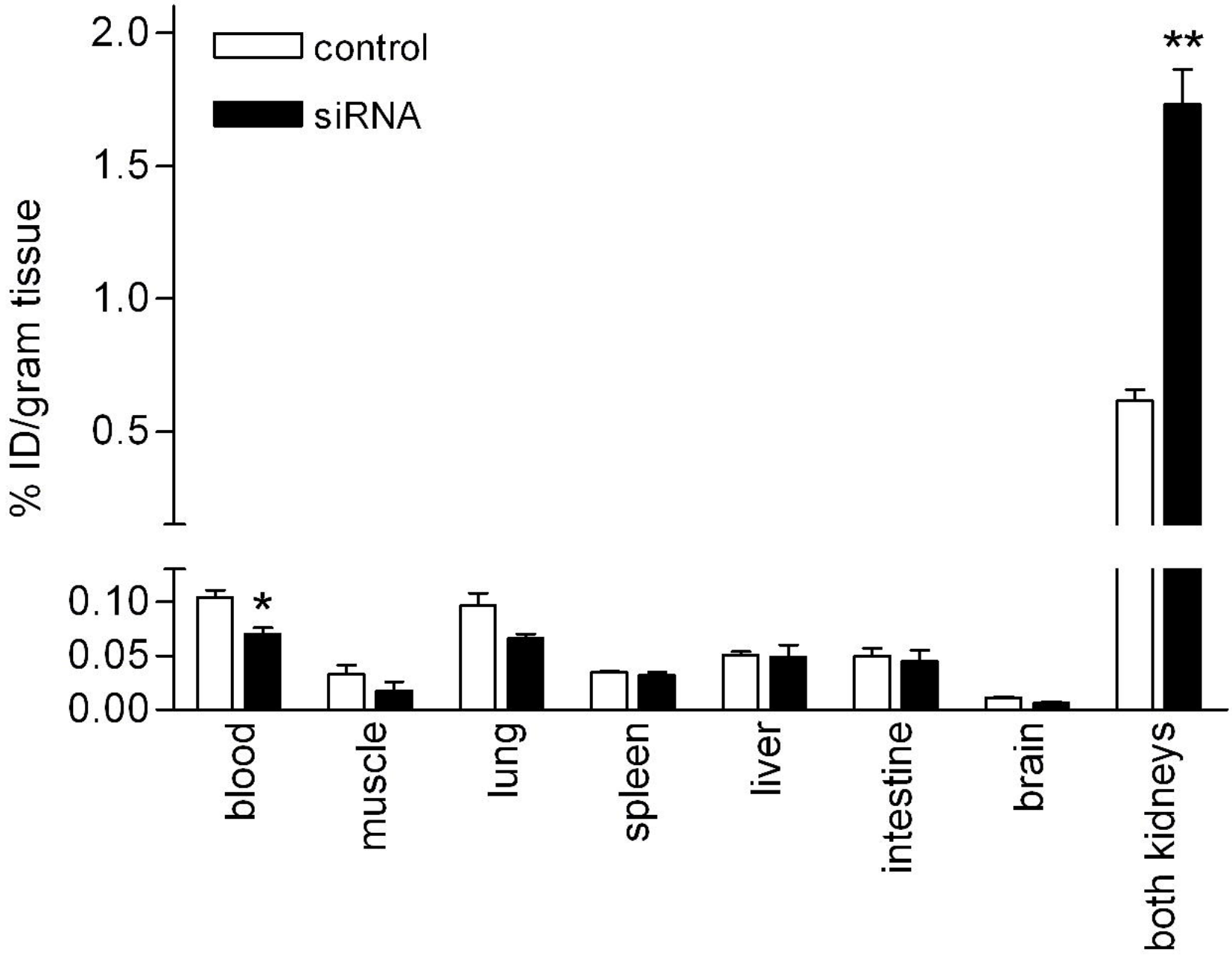
2.0
1.5
1.0
0.5
0.10
0.05
0.00
% ID/gram tissue
control
siRNA
*
**
blood
muscle
lung
spleen
liver
intestine
brain
both kidneys

Figure 2

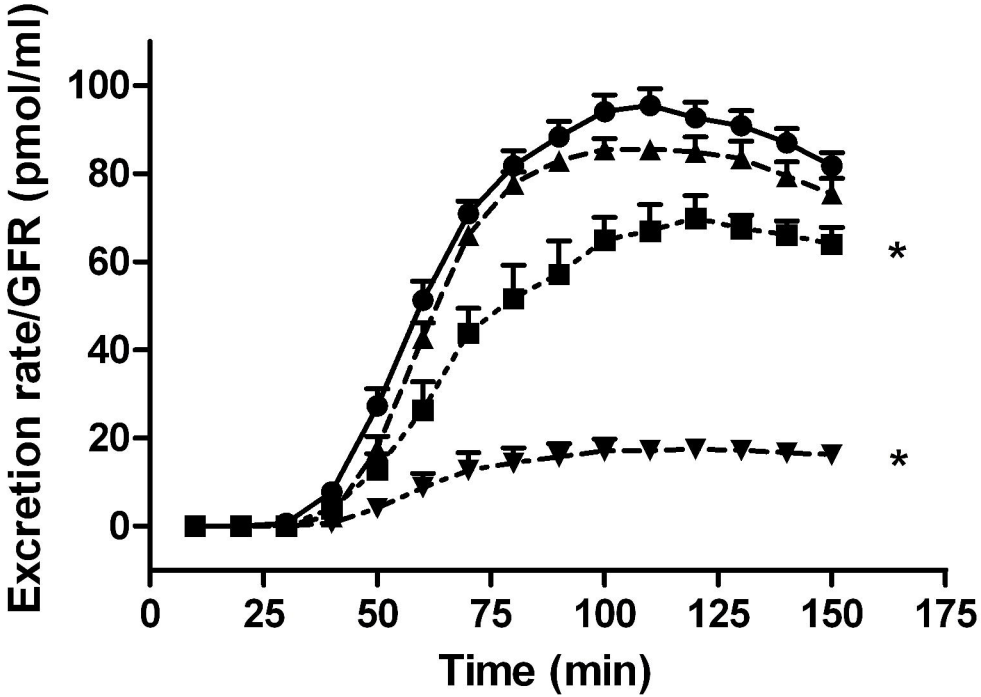
Excretion rate/GFR (pmol/ml)
100
80
60
40
20
0
0
25
50
75
100
125
150
175
Time (min)
*
*

Figure 3