Transcutaneous measurement of renal function in conscious mice

Andrea Schreiber [a], Yury Shulhevich [b], Stefania Geraci [b], Juergen Hesser [b], Dzmitry Stsepankou [b], Sabine Neudecker [b], Stefan Koenig [c], Ralf Heinrich [c], Friederike Hoecklin [d], Johannes Pill [e], Jochen Friedemann [b], Frank Schweda [a], Norbert Gretz [b], Daniel Schock-Kusch [b]

[a] Institute of Physiology, University of Regensburg, D-93040 Regensburg, Germany [b] Institute for Medical Technology of the University Heidelberg and the University of Applied Sciences Mannheim; 68167 Mannheim, [c] Freudenberg Forschungsdienste KG D-69465 Weinheim, Germany, [d] Mektec Europe GmbH D-69465 Weinheim, Germany, [e] Mannheim Pharma & Diagnostics GmbH, 68165 Mannheim Germany

The determination and monitoring of glomerular filtration rate (GFR) is essential when phenotyping animal models or assessing toxicity of novel chemical or medical agents. Especially in mice the measurement of GFR using bolus or constant infusion clearance techniques is difficult and cumbersome. For blood sampling during bolus clearance experiments for instance, the tail or saphenous vein has to be punctured seven times within 75 min. This procedure is stressful for conscious animals, as they have to be restrained repeatedly. Blood sampling without anesthesia, however, is essential to avoid an anesthesia related decrease in GFR.

Here we describe a method permitting the transcutaneous measurement of GFR in conscious mice independent of blood or urin samples. This approach is an extension of our previously published method used in rats. The new technique is based on a miniaturized device that can be mounted on a shaved part of the mice back using double side adhesive tape. It is equipped with an internal memory allowing the transcutaneous measurement of the elimination kinetics of the fluorescent renal marker FITC-sinistrin. This device is described and the validity of the technique in comparison to the plasma clearance is proven in healthy, unilaterally nephrectomized (UNX) and pcy mice.

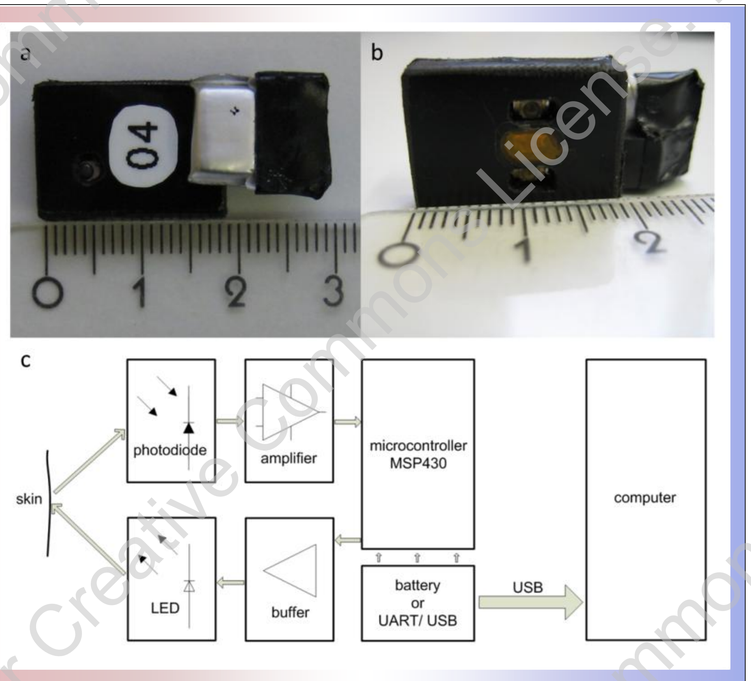

Figure 1: (a) top and (b) bottom view of the miniaturized device for transcutaneous measurement with battery (ruler in cm). (c) Schematic drawing of its building blocks.

$$GFR\ [\mu L/\min/100g\ b.w.] = \frac{14616.8\ [\mu L/100g\ b.w.]}{t_{1/2}(\text{FITC-sinistrin})\ [\min]}$$ equation 1

Equation 1: Conversion of transcutaneously measured excretion $t_{1/2}$ was done using the semi-empirically assessed conversion equation above. The mouse specific conversion factor was established using an equal procedure as previously described for rats, however using two compartment plasma GFR values as basis (1). This leads to a reduced conversion factor, reflecting estimated distribution volume, compared to the factor described for rats (1).

GFR [µl/min/100g bw, mean ± SD]

Mouse model	transcutaneous	two compartment plasma	one compartment plasma (eq.1)
healthy (n=8)	1381 ± 264	1373 ± 182	1212 ± 274
UNX (n=8)	943 ± 189	938 ± 194	883 ± 87
pcy (n=6)	713 ± 207	681 ± 308	712 ± 84

Table 1: Results of the GFR assessments in the three mouse models using the transcutaneous, a two compartment plasma clearance and a one compartment plasma slope only method combined with equation 1 (eq. 1) (healthy and UNX: C57Bl/6 – 129 SV mice; pcy: mouse model of polycystic kidney disease. Plasma and transcutaneous measurements were performed on consecutive days. bw: body weight; SD: standard deviation

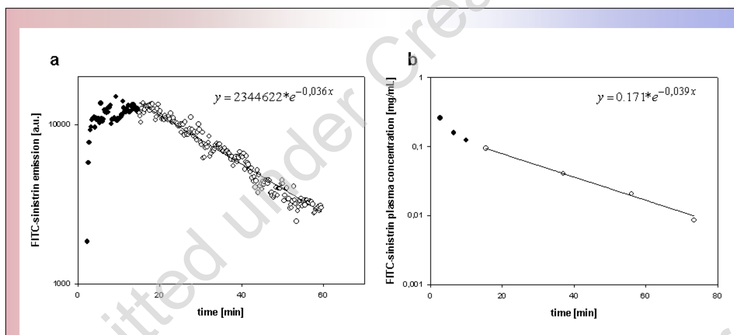

Figure 2: Semi-logarithmic plots of fluorescence signals measured transcutaneously and in plasma after FITC-sinistrin application in mice. A typical example of a transcutaneously measured FITC-sinistrin kinetic with its characteristic increasing phase (closed squares) followed by a single exponential decreasing phase (open squares) is depicted in (a). The black solid line represents the single exponential fitting with its slope α_2 (excretion rate). In respect to the signal-to-noise ratio, the transcutaneous measurement was stopped after 60 min. The corresponding kinetic measured in plasma is given in (b). A fast initial distribution phase (closed squares) is followed by a characteristic single exponential excretion phase. The exponential regression curve is fitted to the slow, single exponentially decreasing part of the curve (solid line) with its slope α_2 (open squares). The measurements in plasma were performed for 75 min in order to replicate established protocols. Dividing Ln(2) by α_2 gives the excretion $t_{1/2}$ of the FITC-Sinistrin.

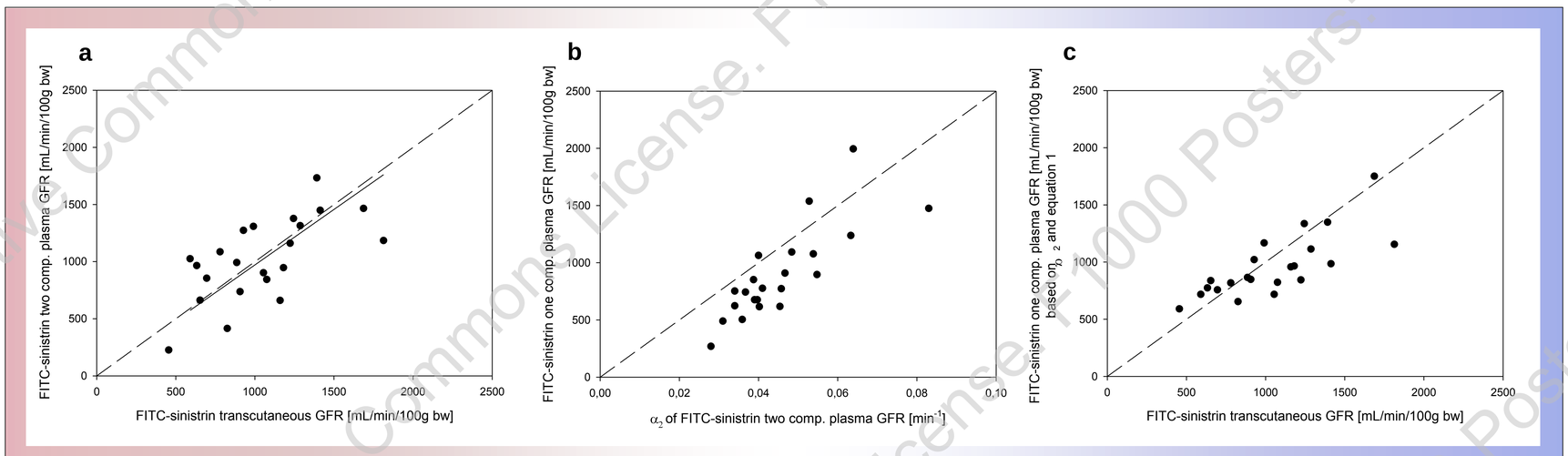

Figure 4: (a) Direct comparison of the GFR values measured on consecutive days with the transcutaneous and the classical two compartment plasma clearance method (dashed line: line of identity; solid line: linear regression with r^2=0.33). (b) Comparison of the rate constant α_2 of the slow, single exponential decay of the two compartment plasma fitting curve vs. a classical one compartment slope-intercept GFR method (dashed line: line of identity; solid line: linear regression with r^2=0.33). (c) Comparison of the transcutaneous GFR and the plasma GFR assessed by $t_{1/2}$ using the introduced conversion factor for both methods (dashed line: line of identity; solid line: linear regression with r^2=0.42). In (a) major differences between the transcutaneous and the two compartment plasma clearance GFR in some animals can be noted. In parts this discrepancy can be assigned to a day to day variability of the GFR. Another factor contributing to the weak correlation might be the strong dose dependence of the classical plasma clearance techniques as GFR = Dose/Area under Curve (AUC). In a one compartment GFR assessment AUC is determined as y-axis intercept of the extrapolated single exponential slow decay part divided by α_2. For this reason a linear behavior between α_2 and one compartment plasma clearance GFR is expected, if the volume of distribution of the animals is comparable. However as seen in (b) the one compartment plasma-clearance GFR values can vary tremendously at virtually equal α_2 values. The dose dependence is especially pronounced in mouse experiments, as extremely small volumes (< 0.1 mL) have to be injected. Therewith minor errors in the injected volume or concentration of the injected fluid can have a huge impact on GFR calculation. By using the same approach for plasma GFR assessment as used for the transcutaneous measurement based on $t_{1/2}$ and the introduced conversion factor this error can be reduced as shown in (c).

In summary: we present a new technique allowing the determination of renal function in freely moving mice without blood or urine sampling as well as without laboratory assays. The technique is validated against a classical plasma clearance method in healthy, UNX and pcy mice.

Further Reading:
1. Schock-Kusch, D. *et al.* Transcutaneous measurement of glomerular filtration rate using FITC-sinistrin in rats. *Nephrol Dial Transplant* 24, 2997-3001 (2009).
2. Schock-Kusch, D. *et al.* Transcutaneous assessment of renal function in conscious rats with a device for measuring FITC-sinistrin disappearance curves. *Kidney Int* 79, 1254-1258, (2011)
3. Schock-Kusch D. et al. Online feedback-controlled renal constant infusion clearances in rats. *Kidney Int* doi: 10.1038/ki.2012.117. (2012)
4. Pill, J. et al. Pharmacological profile and toxicity of fluorescein-labelled sinistrin, a novel marker for GFR measurements. Naunyn Schmiedebergs Arch Pharmacol 373, 204-211 (2006).
5. Pill, J. et al. Direct fluorometric analysis of a newly synthesised fluorescein-labelled marker for glomerular filtration rate. Anal Bioanal Chem 382, 59-64 (2005).
6. Pill, J. et al. Fluorescein-labeled sinistrin as marker of glomerular filtration rate. Eur J Med Chem 40, 1056-1061 (2005).

Contact:
Daniel Schock-Kusch; Medizinische Fakultät Mannheim; Zentrum für Medizinische Forschung Theodor-Kutzer-Ufer 1-3; 68167 Mannheim; daniel.schock-kusch@medma.uni-heidelberg.de