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Transcutaneous assessment of renal function in conscious rats with a device for measuring FITC-sinistrin disappearance curves

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Determination of the urinary or plasma clearance of exogenous renal markers, such as inulin or iohexol, is considered to be the gold standard for glomerular filtration rate (GFR) measurement. Here, we describe a technique allowing determination of renal function based on transcutaneously measured elimination kinetics of fluorescein isothiocyanate (FITC)-sinistrin, the FITC-labeled active pharmaceutical ingredient of a commercially available marker of GFR. A low cost device transcutaneously excites FITC-sinistrin at 480 nm and detects the emitted light through the skin at 520 nm. A radio-frequency transmission allows remote monitoring and real-time analysis of FITC-sinistrin excretion as a marker of renal function. Due to miniaturization, the whole device fits on the back of freely moving rats, and requires neither blood sampling nor laboratory assays. As proof of principle, comparative measurements of transcutaneous and plasma elimination kinetics of FITC-sinistrin were compared in freely moving healthy rats, rats showing reduced kidney function due to unilateral nephrectomy and PKD/Mhm rats with cystic kidney disease. Results show highly comparable elimination half-lives and GFR values in all animal groups. Bland–Altman analysis of enzymatically compared with transcutaneously measured GFR found a mean difference (bias) of 0.01 and a −0.30 to 0.33 ml/min per 100 g body weight with 95% limit of agreement. Thus, with this device, renal function can be reliably measured in freely moving rats eliminating the need for and influence of anesthesia on renal function.

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Glomerular filtration rate (GFR) is the best parameter reflecting overall kidney function. Determination of the urinary or plasma clearance of exogenous renal markers, such as inulin or iohexol, is considered to be as gold standard for GFR measurement.[1] As these procedures are very time consuming and cumbersome, they are neither routinely used in clinical practice nor in the characterization of animal models.[1]

Because of the lack of an easy to handle, precise GFR measurement there are several approaches aiming at the simplification of exogenous clearance procedures.[2,3] Although these techniques still rely on blood and/or urine sampling and laboratory assays. Other experimental approaches are based on the transcutaneous determination of the elimination kinetics of either fluorescent,[4–9] radioactive,[10] or gadolinium-labeled[11] exogenous GFR markers. The major advantages of these procedures are their independence of blood and/or urine sampling and laboratory assays, thus allowing kidney function measurement almost in real time, hence making the detection of rapid changes in renal function (for example, in acute renal failure) possible.[12] A major disadvantage of these techniques in the view of animal experiments, however, is that animals need to be anesthetized during the measurement, resulting in an influence of the anesthesia on renal function.[13,14] Moreover GFR cannot be determined by classical single or multiple compartment models, as only relative changes in fluorescence, radiation, or magnetic resonance contrast can be determined, but not total concentrations. Hence, these techniques are restricted to the determination of the rate constant (m) or excretion half-life ($=\ln_2/m$) of the latter single exponential excretion phase of the exogenous markers. It has been shown, that this rate constant is a very close estimate of GFR/extracellular volume space, therewith

being a valid parameter reflecting renal function.[15] The theoretical background of this fact is beyond the scope of this Technical Note, the interested reader is referred to recent literature.[15]

In this Technical Note, we focus on a novel device for the transcutaneous measurement of the elimination kinetics of the fluorescent-labeled exogenous GFR marker fluorescein isothiocyanate (FITC)-sinistrin[7,16,17] in freely moving rats.

DEVICE

The miniaturized and flexible instrument is built up from a light-emitting diode with an emission maximum for FITC at 480 nm (ELS-525-892 EPIGAP Optoelektronik GmbH, Berlin, Germany) and a photodiode that detects the emitted light at 520 nm (EPD-525-1-0.9-1 EPIGAP Optoelektronik GmbH). To select the appropriate wavelength, a blue resp. green bandpass filter is placed in front of the light-emitting diode and photodiode (071 Tokyo Blue, <500 nm, 015 Deep Straw, >480 nm, Lee Filters, Andover, England). After highly sensitive amplification and digitization (10 bit), the data are transferred over radio frequency (EZ430-RF2500, Board Kit, MSP430, Texas Instruments) to a remote PC, in which the kinetic parameters are determined. For use on awake rats, the local energy is supplied by a small accumulator battery. The optical part of the device and its building blocks are shown in Figure 1.

FITC-Sinistrin

Sinistrin is the active pharmaceutical ingredient of the commercially available GFR marker Inutest (Fresenius Kabi, Linz, Austria). The labeling reaction has been described elsewhere.[16,17]

PROOF OF PRINCIPLE

Simultaneous measurements of transcutaneous and plasma elimination kinetics of FITC-sinistrin were performed in freely moving healthy rats ($n=7$) and rats having reduced kidney function due to unilateral nephrectomy ($n=5$) or cystic kidney disease (7 months old PKD/Mhm rats,[18] $n=6$). The electronic part is incorporated into a rodent jacket (Lomir Biomedical, Malone, NY), whereas the optical components are fixed on a depilated region on the back of the rat using a sticky patch (Lohmann GmbH KG, 56567, Neuwied, Germany). All animal experiments were performed according to international and local regulations/guidelines equivalent to the NIH Guide for the Care and Use of Laboratory Animals.

For the experiment the animals were prepared, as described previously.[7,16,17] After a baseline period, where the system detects a constant background signal, FITC-sinistrin (63 mg per 100 g body weight (b.w.)) was injected via a catheter into the left femoral vein. The sampling rate of the device was 60 measurements per min, the excitation time lasted 10 ms per measurement and the whole experiment was performed over 120 min resulting in 7200 data points/session (Figure 2a). The FITC-sinistrin elimination kinetics were simultaneously assessed transcutaneously, as well as fluorometrically in plasma samples. Moreover, the fructan moiety of the FITC-sinistrin was determined enzymatically in plasma samples as a gold standard technique of GFR measurement, as published before.[7,16,17,19] From the plasma samples the rate constant and elimination half-life were calculated using an established one compartment model (Figure 2b).[7,16,17] In addition, a two compartment model was fitted to the transcutaneous data for half-life determination (see Supplementary Data online).

Figure 2 shows representative excretion kinetics determined transcutaneously as well as fluorometrically and enzymatically in plasma.

GFR was calculated out of the derived half-lifes and a semi-empirical approach (validated in rats) using a formula published before:[7]

$$GFR\,[ml/\min/100g\ b.w.] = \frac{31.26\,[ml/100g\,b.w.]}{t_{1/2}\,(\mathrm{FITC-sinistrin})[\min]} \quad (1)$$

COMPARISON OF MEASUREMENTS OBTAINED TRANSCUTANEOUSLY AND IN PLASMA

Transcutaneous and plasma measurements were tolerated well by the animals. The results of the simultaneously performed techniques are given in Table 1. Overall, the mean

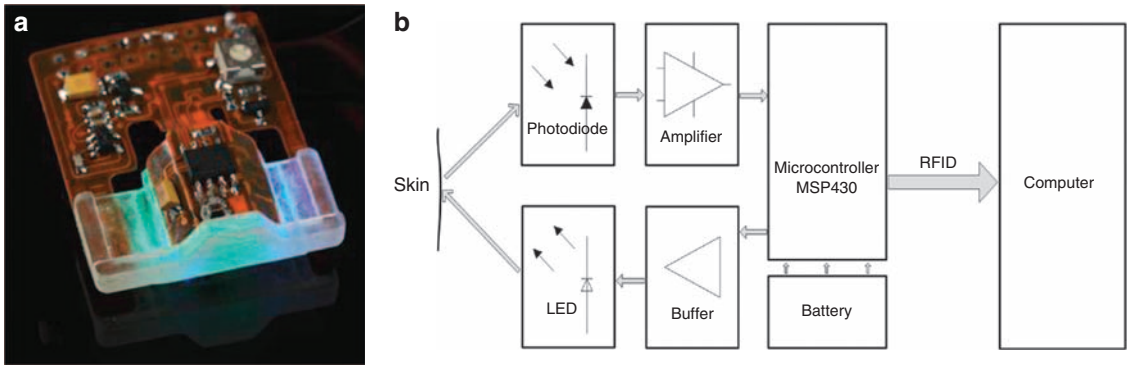

Figure 1 | New device. Optical part of the new device for transcutaneous measurement (**a**) and a schematic drawing of its building blocks (**b**). LED, light-emitting diode; RFID, radio-frequency identification.

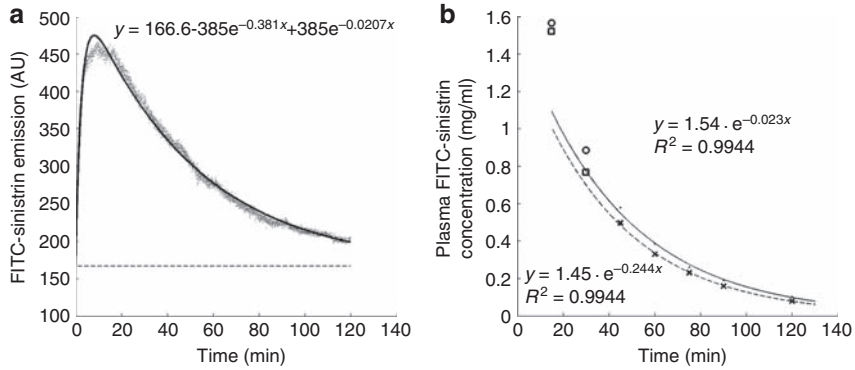

Figure 2 | Excretion kinetics. Example of a transcutaneously (**a**) measured disappearance curve and its counterpart measured in plasma enzymatically as well as fluorometrically (**b**) after intravenous injection of 63 mg per 100 g body weight fluorescein isothiocyanate (FITC)-sinistrin dissolved in phosphate-buffered saline (400 mg/ml). (**a**) Shows a typical example of a transcutaneously measured FITC-sinistrin excretion kinetic and the fitted bi-exponential regression curve. The background signal of the measurement of 166.6 AU is plotted as dashed line. (**b**) Gives the corresponding excretion kinetics measured in plasma and the exponential regression curve fitted to the second part of the curve (black data points, solid line represents fluorometric measurement; black crosses, dashed line represents enzymatic measurement). Open circles (fluorometric) and open squares (enzymatic) indicate the initial fast decrease in concentration in plasma caused by the combination of diffusion into the interstitial space and the renal clearance of FITC-sinistrin.

Table 1 | Comparison of kinetic results and GFR determination of FITC-sinistrin in three different rat models

	Enzymatic measurement (plasma) mean ± s.d.				Transcutaneous measurement mean ± s.d.				Fluometric measurement (plasma) mean ± s.d.			
Animal group	GFR (ml/min per 100g b.w.)	$t_{1/2}$ (min)	95% CI low	95% CI high	GFR (ml/min per 100g b.w.)	$t_{1/2}$ (min)	95% CI low	95% CI high	GFR (ml/min per 100g b.w.)	$t_{1/2}$ (min)	95% CI low	95% CI high
Healthy s.d. (*n*=7)	1.2 ± 0.1	26.5 ± 1.5	23.8 ± 3.1	30.8 ± 2.1	1.2 ± 0.2	27.3 ± 3.9	27.2 ± 3.9	27.4 ± 3.9	1.2 ± 0.1	25.3 ± 1.5	20.9 ± 1.5	34.2 ± 4.0
UNX s.d. (*n*=5)	0.9 ± 0.04	33.5 ± 1.5	28.9 ± 1.7	40.2 ± 5.5	0.8 ± 0.3	34.8 ± 4.7	34.5 ± 4.8	35.0 ± 4.7	0.9 ± 0.03	35.8 ± 1.1	31.4 ± 1.1	41.8 ± 3.6
PKD/Mhn (*n*=6)	0.9 ± 0.1	33.3 ± 3.3	29.6 ± 3.8	37.1 ± 5.2	0.9 ± 0.2	34.0 ± 6.7	33.7 ± 6.5	34.4 ± 6.8	0.9 ± 0.1	36.7 ± 3.4	33.5 ± 3.1	40.9 ± 4.2

Abbreviations: b.w., body weight; CI, confidence interval; FITC, fluorescein isothiocyanate; GFR, glomerular filtration rate; $t_{1/2}$, half-life.
The 95% confidence intervals of the derived half-lifes from the enzymatic and fluorometric (plasma) measurement show a wide range of about 7–10 min, the 95% confidence intervals of the transcutaneously derived half-lifes exhibit only a range of <1 min. This is due to the much more reliable regression analysis through the high number of data points. It indicates a correspondingly higher precision of the transcutaneous measurement compared with the enzymatic and fluorometric measurements in plasma.

half-life and GFR values are compared favorably. Figure 3 reveals the correlation of the simultaneously derived enzymatically and transcutaneously derived excretion half-lifes and GFR values. The precision of the measurements is depicted by a Bland–Altman plot.[20,21]

An interesting finding is that the 95% confidence intervals of the transcutaneous method are significantly narrower compared with the 95% confidence intervals of the measurements in plasma, but the standard deviation of the plasma measurements within the animal groups are lower compared with the transcutaneous measurements (Table 1).

The 95% confidence interval is a measure of the quality of the curve fitting procedure. As the number of measurement points in the transcutaneous measurement is much higher compared with the blood sample measurement, the 95% confidence intervals of the transcutaneous measurement has to be narrower compared with the blood measurement for mathematical reasons. However, this implies that because of the more robust curve fitting procedure the transcutaneous method might be more sensitive than the plasma measurement. This higher sensitivity of the transcutaneous method on the other hand can lead to a higher standard deviation within the animal groups, and therewith limited precision in the Bland–Altman plot as well as weak correlation parameters, like r^2, in the comparison of the methods. The reason for this is that differences in GFR might be detected by the transcutaneous method that are not reflected by the plasma measurements due to its poorer sensitivity. However, there are also still issues, which have to be further investigated that might influence the reliability of the transcutaneous method like differences in skin thickness, influence of altered skin perfusion by gluing the plaster to the skin, or influence of the skin penetration depth of the light beam. The limited number and size of the animal groups is a further drawback in this proof of concept study.

RATE CONSTANT VERSUS GFR

In rats, either the rate constant or half-life itself can be used as measure of kidney function. Moreover, the half-life can be converted into GFR by an empirically derived conversion factor[8] or by curve fitting procedures in combination with an estimated plasma volume.[9] A drawback of the conversion of the rate constant into GFR is that the distribution volumes need to be assumed stable. This might lead to errors in some experimental settings in which edema and changes in GFR occur at the same time. An advantage of the rate constant as measure of renal function is its independence in situations, wherein extracellular volume space changes, but GFR is

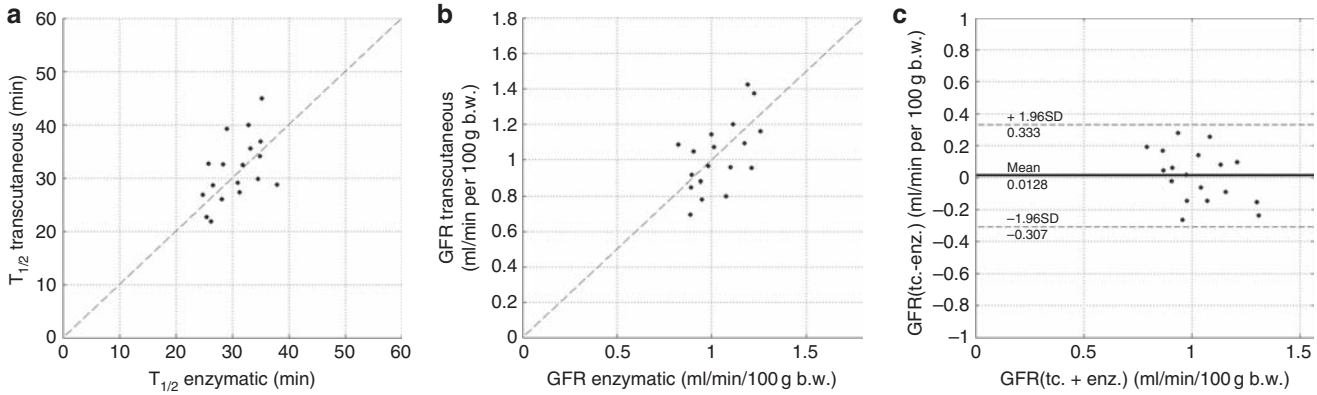

Figure 3 | Method comparison for determination of half-life and glomerular filtration rate (GFR) in rats after intravenous injection of FITC-sinistrin measured transcutaneously as well as enzymatically in plasma. Scatter plot of half-life ($r^2 = 0.57$ for $y(x) = ax$ regression function) (**a**) and calculated GFR ($r^2 = 0.59$ for $y(x) = ax$ regression function) (**b**) with lines of identity (dashed), are depicted ($n = 18$). Bland–Altman plot (**c**)[20,21] shows a mean difference (bias) of 0.01 ml/min per 100 g b.w. and a 95% limit of agreement of −0.30 to 0.33 ml/min per 100 g b.w.

unaffected. In such a situation, classical bolus clearance techniques may show an apparent rise or decline in GFR.[15] An additional advantage of the indexation of GFR to extracellular volume space is its technical simplicity, as only the rate constant needs to be determined,[15] which as a parameter is independent of dosage or injection errors.

COMPARISON TO OTHER APPROACHES

Other approaches have been published only allowing the transcutaneous measurement of kidney function in anesthetized rodents using fluorescent-labeled functional markers, in combination with a fluorescent microscope or laser-based measuring devices.[4–9]

Recently, Wang *et al.*[9] introduced a ratiometric fluorescent technique based on the measurement of a reporter and a marker substance using a fluorescent microscope and a two compartment mathematical model in anesthetized rats. The use of a ratiometric technique to overcome movement artifacts is of high interest especially in measurements in freely moving animals. However, the injection of a reporter molecule showing an excretion half-life close to infinity is not suitable for human use, especially with respect to toxicity.

POTENTIAL FOR HUMAN APPLICATION

The described method has the potential to become a convenient method for precise renal function measurement in humans. It would allow monitoring renal function in populations at high risk of developing chronic kidney failure, similar to diabetic or hypertensive patients, predominantly in the near normal range, when e-GFR gives unclear results.[1] Other possible applications could be GFR monitoring of patients in intensive care units with a high risk of developing acute renal failure,[11] or for dose adjustment of renally excreted or nephrotoxic drugs, which is especially crucial in children, elderly, and patients receiving anticancer drugs.

The limitation of the method concerning the determination of total GFR could be overcome in humans by a formula

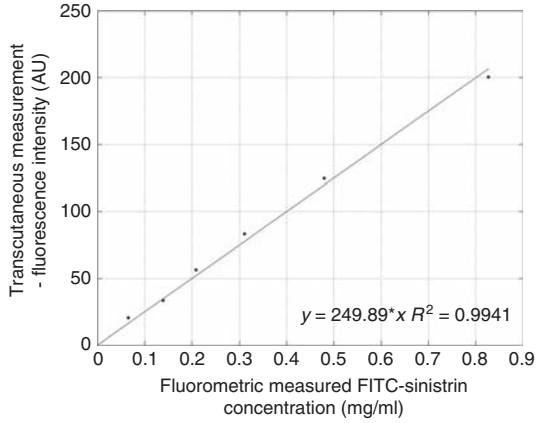

Figure 4 | Scatter plot of transcutaneously measured emission signal and fluorometrically measured fluorescein isothiocyanate (FITC)-sinistrin concentration in plasma. The plot illustrates that a transcutaneous measurement is possible down to a plasma concentration of about 0.07 mg/ml.

derived by Bird *et al.*[22] allowing the estimation of extracellular volume space from the patients' weight and height. This equation makes the calculation of total GFR from the rate constant possible.[11,22]

For human use, the fluorescence signal per mg substance and the sensitivity of the device has to be improved to reach a suitable dosage for humans. With the described settings it is possible to transcutaneously measure FITC-sinistrin concentrations down to 0.07 mg/ml plasma concentration (Figure 4).

IN SUMMARY

We present a device allowing the determination of renal function in freely moving rats with no need for blood sampling and laboratory assays. Such a method is valuable in studies in which effects of anesthesia on GFR have to be avoided. Because of its independence from blood sampling,

multiple/repetitive GFR determinations can be performed within a short-time period in the same animal. Compared with classical plasma clearance techniques the robustness of the new technique is demonstrated in animal models without and with renal impairment.

DISCLOSURE

DS-K, MS, JP, JH, and NG are inventors on patents and patent applications covering this subject. YS was supported by a grant from Fresenius Medical Care AG KGaA. The remaining authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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