

# Real-Time Point-of-Care Measurement of Impaired Renal Function in a Rat Acute Injury Model Employing Exogenous Fluorescent Tracer Agents

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## Introduction

Kidney function assessment is needed for the detection and treatment of acute kidney injury and chronic kidney disease. Kidney function is most commonly assessed by determining glomerular filtration rate (GFR) which is achieved by measuring the clearance of a tracer such as Inulin that is freely filtered by the kidneys, does not undergo extrarenal elimination, is not secreted nor reabsorbed by the renal tubules and is not metabolized or degraded. Current tracers used to measure renal function lack sensitivity (i.e. serum creatinine), or are cumbersome to measure. An exogenous GFR fluorescent tracer, whose elimination rate could be monitored non-invasively through skin would provide a substantial improvement over any currently available method. We developed a series of novel aminopyrazine analogs for use as exogenous fluorescent GFR tracers that emit light in the visible region for monitoring GFR non-invasively over skin. Recently, we showed that many of these compounds met the criteria of GFR tracers. In rats, these compounds are eliminated by the kidney with urine recovery greater than 90% of injected dose, are not broken down or metabolized *in vivo*, are not secreted by the renal tubules, and have clearance values similar to a GFR reference compound, iothalamate. In addition, biological half-life ( $\ln(2)/\text{Elimination Rate Constant}$ ) of these compounds measured in rats by non-invasive optical methods correlated with plasma derived methods. In the current study, we continued our evaluation of PP-2338, a 2338 Dalton pegylated pyrazine compound. We previously showed that PP-2338 met criteria as a GFR tracer in normal rats and measured the elimination rate constant (biological half life) using noninvasive optical methods. However, we did not know if these methods could detect impaired renal function. Therefore, we evaluated PP-2338 in a 5/6th nephrectomy acute kidney injury rat model. Our hypothesis is that acute kidney injury resulting in impaired renal function will increase optically derived biological half life of PP-2338 *in vivo* in a 5/6th nephrectomy rat model.

## Methods

**Animals:** Acute kidney injury was induced in anesthetized male Sprague-Dawley Rats by performing a 5/6 Nephrectomy. Rats were anesthetized with Isoflurane (1-2%) and prepared for survival surgery. The left kidney was isolated from surrounding fat and the renal artery exposed. The lower and upper branch of the renal artery were isolated and tied off with suture. The central branch was left alone and patent. The right kidney was then isolated from surrounding fat and completely tied off and removed from the rat. Sham rats were handled in the same manner without tying off the left renal arteries or removing the right kidney.

**Tracer:** PP-2338 is a pyrazine conjugated with 2 PEG side chains. The molecular weight is 2338 with absorption and emission maxima around 450 nm and 560 nm respectively.

**Experimental Design:** To test whether acute kidney injury will significantly increase optically derived half-life of PP-2338 in rats, we measured optically derived biological half life of PP-2338 in 5/6th nephrectomized rats and surgical Sham rats over a time course of 4, 12, 24, 48, 72 hours and 1 week post injury. Sixteen rats were randomized into 5/6th Nephrectomy and Sham groups. Serum creatinine levels were measured at each time point as an established reference of renal function. At 1 week post injury, a plasma pharmacokinetic study was performed with PP-2338 to obtain clearance, terminal half-life, and volume of distribution. Plasma pharmacokinetic parameters were compared to serum creatinine and optical derived measures of renal function.

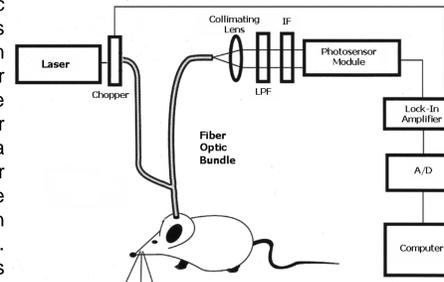
**Plasma Pharmacokinetic.** Blood samples were collected at 1, 6, 12, 18, 30, 45, 60, 90 and 120 minutes post injection by an arterial catheter. Pharmacokinetic parameters analyzed using WinNonLin PK modeling software.

**Measurement of tracer, serum creatinine and determination of pharmacokinetic parameters:** The quantification of PP-2338 in plasma was performed via HPLC fluorescence detection using calibration standards created from the dosing solution. Serum creatinine was measured on a HESKA Blood Chemistry machine. Pharmacokinetic parameters were determined from plasma concentrations using WinNonLin pharmacokinetic modeling software.

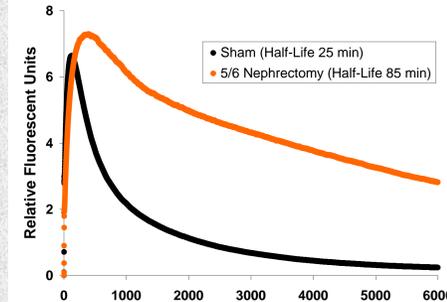
**Optical Renal Function Studies:** One ear lobe of an anesthetized rat was glued flat to a glass slide positioned approximately 2 mm beneath a fiber optic bundle connected to an optical system for recording fluorescence as shown in Fig. 1. The distribution and elimination of PP-2338 was monitored at the ear with the fiber optic system. Terminal Half-life of PP-2338 was determined from the elimination curve analyzed with WinNonLin pharmacokinetic modeling software.

## Results

**Fig. 1.** A 445 nm solid state laser source (Power Technology model LDCU12/6619) directed through a chopper (Stanford Research Systems model SR540) and into one leg of a silica bifurcated fiber optic bundle (Oriol #77565). The common end of this bifurcated bundle was placed approximately 2 mm from the rat ear. The second leg of the bifurcated fiber optic bundle was fitted with a collimating beam probe (Oriol #77644) and placed in front of a photodetector (Hamamatsu photosensor module H7827-011) with a long pass filter and a narrow band interference filter (Semrock LP02-488RS-25, FF01-560/25-25). The output of the photosensor was connected to a lock-in amplifier (Stanford Research Systems model SR830). The lock-in output was digitized (National Instruments NI-USB-6211) and the digitized data was acquired by computer using LabVIEW data acquisition software.



### Representative Elimination Curve and Running Half Life of PP-2338 Measured Optically in a Rat



**Fig. 2.** Representative elimination curves of PP-2338 measured optically in anesthetized 5/6 Nephrectomized and SHAM rats 7 days post injury. Elimination of PP-2338 is severely reduced in the 5/6<sup>th</sup> nephrectomy group. Biological half-life of PP-2338 in the rat was calculated from the negative log slope of the curves during the elimination phase (2000 - 6000 seconds).

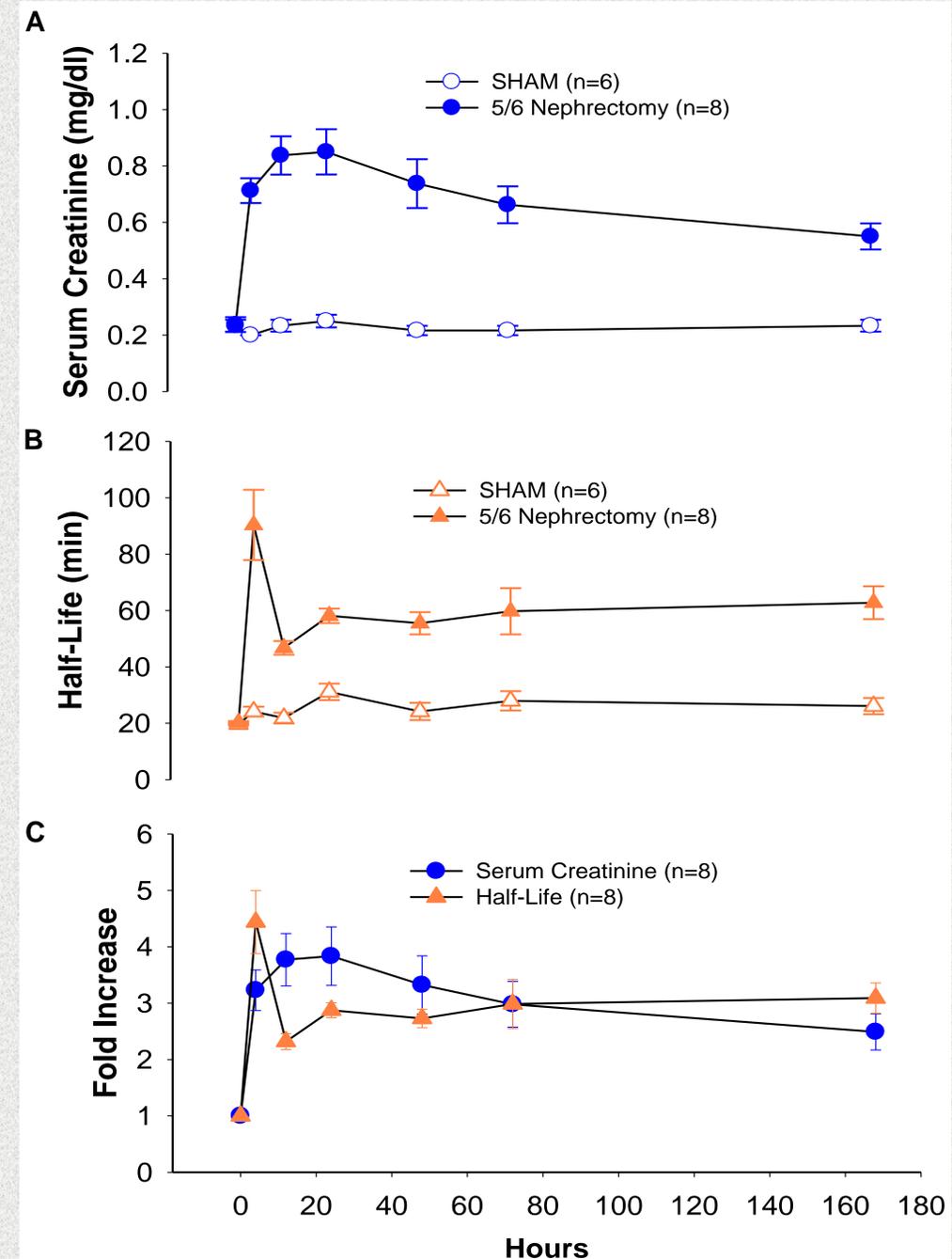
**Table 1. Comparison of PP-2338 Pharmacokinetic Parameters and Serum Creatinine in 5/6 Nephrectomized and SHAM Rats 1 Week Post Injury**

	CL (mL/min)	V <sub>ss</sub> (ml)	t <sub>1/2</sub> (min) Plasma	t <sub>1/2</sub> (min) Optical	Serum Creatinine (mg/dl)
Sham (n=6)	2.1 ± 0.1	57 ± 2	27 ± 1	26 ± 3	0.2 ± 0.1
5/6 Nephrectomy (n=8)	1.0 ± 0.1*	63 ± 4	51 ± 5*	63 ± 6*	0.6 ± 0.1*

On day 7 post injury, clearance (CL) of PP-2338 was significantly reduced in 5/6 nephrectomy, suggesting this tracer is useful in detecting renal impairment. Renal dysfunction was confirmed by a significant elevation of serum creatinine. Plasma and optical derived half-lives (t<sub>1/2</sub>) were similarly increased in the nephrectomy group compared to the SHAM group. Volumes of distribution (V<sub>ss</sub>) were not significantly different between the treatment groups. \*P < 0.05, t-Test.

## Summary

- Acute kidney injury induced by 5/6 Nephrectomy resulted in impaired renal function as measured by elevated serum creatinine levels, decreased clearance and increased half-life of PP-2338.
- Optical derived half-life of PP-2338 was effective in detecting impaired renal function
- Renal function as a measure by the half-life of PP-2338 was more dynamic than serum creatinine.



**Figure 3.** Time course of (A) Serum Creatinine, (B) Half-Life of PP-2338, and (C) Fold increase in serum creatinine and Half-Life of PP-2338 in Nephrectomized and SHAM rats. Nephrectomy increased both serum creatinine and half-life of PP-2338 which remained elevated throughout the 7 days post injury. Peak serum creatinine levels were reached 24 hours after nephrectomy, while peak PP-2338 Half-Life was achieved in only 4 hrs. In the first 48 hours, half-life of PP-2338 was more dynamic than serum creatinine levels. From 48 hours to 1 week, the nephrectomy induced fold increase in serum creatinine and PP-2338 half-life was stable and similar.