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A PET Tracer For Renal Organic Cation Transporters, ^{11}C-metformin: Radiosynthesis and Preclinical Proof-of-Concept Studies

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Short running foot line: ^{11}C-metformin as a renal PET tracer

ABSTRACT

Organic cation transporters (OCT) in the kidney proximal tubule (PT) participate in renal excretion of drugs and endogenous compounds. PT function is commonly impaired in kidney diseases and consequently quantitative measurement of OCT function may provide an important estimate of kidney function. Metformin is a widely used drug and targets OCT-type 2 (OCT2) located in PT. Thus, we hypothesize that 11C-labeled metformin would be a suitable PET tracer for quantification of renal function.

Methods: 11C-metformin was prepared by 11C-methylation of 1-methylbiguanide. *In vitro* cell uptake of 11C-metformin was studied in LLC-PK_1 cells in the presence of increasing doses of unlabelled metformin. *In vivo* microPET studies in Sprague Dawley rats were performed at baseline and after treatment with OCT-inhibitors to evaluate renal uptake of 11C-metformin. Kidney and liver pharmacokinetics of 11C-metformin was investigated *in vivo* by dynamic 11C-metformin PET/CT in six anesthetized pigs, and renal clearance of 11C-metformin was compared with renal clearance of 51Cr-EDTA. Formation of 11C-metabolites was investigated by analysis of blood and urine samples.

Results: The radiochemical yield of 11C-metformin was 15 ± 3% (*n = 40*, decay-corrected) and up to 1.5 GBq tracer was produced with a radiochemical purity >95% in less than 30 min. Dose-dependent uptake of 11C-metformin in LLC-PK_1 cells was rapid. Rat microPET images showed 11C-metformin uptake in kidney and liver, the kinetics of which was changed after challenging animals with OCT inhibitors. In pigs, 80% of the injected metformin dose was rapidly present in the kidney and a high dose of metformin caused a delayed renal uptake and clearance compared to baseline consistent with transporter-

mediated competition. Renal clearance of 11C-metformin was approximately 3 times the renal clearance of 51Cr-EDTA.
Conclusion: We have successfully synthesized a novel 11C-metformin tracer and PET studies in rats and pigs showed rapid kidney uptake from the blood and excretion into the bladder similar to other radiopharmaceuticals developed for gamma-camera renography.

Key Words: Renal function; Organic cation transporter; Positron emission tomography; Glomerular filtration rate; Metformin

INTRODUCTION

Organic cation transporters (OCTs) in the kidney, liver, intestine, brain, and placenta play essential physiological and pharmacological roles in the handling of cationic drugs and endogenous organic ions (*1*). In the kidney, human OCTs and multidrug and toxin extrusion proteins (MATEs) are the major transporters responsible for the secretion of cationic drugs into the urine. Specifically, OCT type-2 (OCT2) mediates the uptake of drugs from the blood at the basolateral membrane of the proximal tubular epithelial cells, and MATE1 and MATE2K secrete drugs from cells into the lumen of proximal tubules (*1*,*2*). Creatinine is an important OCT2 substrate, which may explain why medication with cationic drugs can influence tubular secretion of creatinine. Consistent with this, a recent study demonstrated that variants in the gene SLC22A2, encoding OCT2, is associated with phenotypes of net tubular creatinine secretion and end stage renal failure (*3*). A downregulation of OCT2 was associated with an experimental acute renal failure in rats (*4*,*5*). This indicates that OCT2 may play an important role in renal disease. It was recently demonstrated that OCT2 is important for renal metformin transport, and other cationic drugs may competitively inhibit the renal metformin transport (*6*).

Consequently, a non-invasive method to assess the renal transport of cationic compounds would be of considerable interest given its potential to improve our understanding of normal renal physiology, pathophysiology, and the renal effects and/or side effects of new drugs. There have been a few attempts to develop a renal cationic tracer for gamma-camera studies however they have not advanced into the clinic (*7*). Since some of the currently available common methods for measuring kidney function (such as renography with 99mTc-MAG3) also is based on proximal tubule transport by another transport system (organic anion transporters) there may be an interest in developing an alternative method for measuring kidney function with radiotracers transported by OCTs.

Recently in this journal, PET/CT was used in conjunction with 68Ga-EDTA to measure the glomerular filtration rate (*8*), suggesting that PET/CT is a suitable methodology to also study the renal and hepatic handling of cationic drugs, but so far no specific cation tracer has emerged for renal PET studies. The ideal tracer has high affinity and is specific for the OCT2 transporter, has negligible metabolism and plasma protein binding with a high first-pass renal extraction, and is excreted in high concentrations in the urine.

We therefore hypothesized that the diabetes drug metformin, labelled with carbon-11 ([N-methyl-^{11}C] metformin (11C-metformin)), is a suitable PET tracer for quantitative PET/CT studies of renal metformin excretion and hence provide a direct measurement for organic cation transport properties in the proximal tubule. The aim of this study therefore is to examine if 11C-metformin can be used as a radiotracer for renal PET imaging studies. Here we present the radiosynthesis of 11C-metformin, in vitro studies in kidney cells and preclinical PET studies in rats. Exploratory proof-of-concept PET/CT studies in anesthetized pigs included dynamic PET/CT of the kidney and liver and analysis for ^{11}C-metformin in the blood and urine. Competition between ^{11}C-metformin and other compounds for the organic cation transporters was investigated. Additionally, the biodistribution of 11C-metformin was also determined.

MATERIALS AND METHODS

Radiochemistry

^{11}C-metformin was prepared by the radiosynthesis as illustrated in Fig. 1 and described in detail in the supplemental information. The radiochemical purity of 11C-metformin was determined by analytical HPLC (Please see the supplemental information).

In vitro and *in vivo* studies

The study comprised a series of *in vitro* and *in vivo* studies using rats and pigs. A summary of the pig experiments is shown in Table 1. For detailed description, please see supplemental information.

RESULTS

Radiochemistry

The one-step radiosynthesis provided 0.4-1.5 GBq of 11C-metformin within 30 min with a radiochemical yield of 15 ± 3% (mean ± SD; n = 40, decay-

corrected) and a radiochemical purity greater than 95%. In its final formulation (0.1-0.5 μg/mL metformin in aqueous 100 mM $(NH_4)_2HPO_4$; pH 5), the tracer showed no alterations in pH, chemical or radiochemical purity for up to 1.5 h after the end of the synthesis.

In vitro cell experiments

LLC-PK_1 cells displayed linear time-dependent uptake of 11C-metformin that could be challenged by prior treatment with unlabelled metformin (Fig 2A). Reincubation of tracer-loaded LLC-PK_1 cells in tracer-free medium showed a mono-exponential disappearance of 11C-metformin to approximately 60% after 30 min. The clearance rate was estimated as $k = 1\ hour^{-1}$.

Autoradiography studies

Figure 3 shows representative images of 11C-metformin uptake in the liver and kidneys from rats 2 min post tracer injection. In the liver there is a homogenous distribution of 11C-metformin (Fig 3A) which is much weaker than 11C-metformin uptake in the kidney (Fig 3B). In the kidney the uptake is heterogeneous with relative strong uptake in the cortical regions and with an even more intense uptake corresponding to the medullary regions of the kidney.

In Vivo Studies

MicroPET experiments in rats

To examine the pharmacokinetic properties of 11C-metformin, rats were scanned after iv administration of the tracer. The dynamic PET recordings demonstrated a rapid uptake of 11C-metformin into the kidney and less pronounced into liver tissue (Fig 4A and B). With regard to the renal uptake, it is noteworthy that the renal cortex shows a characteristic 3 phase pattern similar to a classical renogram with a rapid phase 1 lasting a few seconds corresponding to the arterial input of a bolus passage of 11C-metformin to the kidney and thereby representing the renal blood flow. In phase 2, 11C-metformin accumulates from 0.5 min to 2 min in the kidney in proportion with the functional capacity of the renal parenchyma. Excretion from renal cortex dominates beyond 2 min in phase 3. Characteristically the liver uptake is markedly lower than the renal uptake.

To examine the drug interaction properties of OCT in the kidney and liver, rats were subjected to PET imaging with 11C-metformin after iv administration of cold metformin, tetrabutylammonium hydrogensulphate (TBA), cimetidine, cisplatin and quinine (Fig. 4D and E). These drugs were chosen with diversity

in mind, based on their reported affinity for OCTs and MATEs. Metformin and cimetidine reduced the maximum renal cortex uptake of 11C-metformin slightly, whereas the whole kidney Standard Uptake Value (SUV) was not affected. Cisplatin and quinine had a more pronounced effect on cortex TAC, along with a markedly higher kidney SUV. TBA administration caused an irreversible retention of 11C-metformin in renal cortex accompanied by a large increase in kidney SUV. Only TBA and cimetidine caused the liver SUV to increase compared to baseline value.

PET/CT experiments in pigs

Similar to what was seen in rats, dynamic PET recordings in pigs demonstrated a rapid uptake of 11C-metformin into the kidney and a less pronounced uptake into liver tissue (Fig 5A and B). Renal uptake markedly exceeded liver uptake. Coronal slices of summed PET images at various times after tracer injection (Fig 5A) show a homogenous distribution and rapid uptake of 11C-metformin in the renal cortical parenchyma within the first minute. In the succeeding images the distribution of 11C-metformin is seen to concentrate in the renal medulla demonstrating excretion of 11C-metformin. Again, the dynamic PET recordings show the characteristic 3 phase pattern similar to a classical renogram, as already observed in rats. Administration of cold metformin had no

effect on the plasma profile of 11C-metformin yet resulted in lower renal cortex radioactivity and slower liver wash-out, compared to baseline. From the pharmacokinetic analysis of 11C-metformin in the renal cortex, we calculated rate constants K_1 and k_2, along with distribution volumes (Table 2). The plasma-to-urine clearance could be estimated to 382 ml/min at baseline, dropping to 179 ml/min after metformin challenge.

Clearance studies

To examine the magnitude of metformin clearance compared with the glomerular filtration rate we measured the urine clearance of 11C-metformin and 51Cr-EDTA in 2 pigs (Table 3). The urine clearance of 11C-metformin was 3.3 times higher than the 51Cr-EDTA urine clearance, with similar baseline 11C-metformin clearance values as estimated from the pharmacokinetic analysis.

Biodistribution and dosimetry

The biodistribution of 11C-metformin was examined in a 40 kg female pig at 5 different time points (Fig 6). The primary sites of radioactivity accumulation were kidney, liver, salivary glands and urinary bladder wall (small intestines became visible 60 min post injection), whereas the brain was devoid of

radioactivity. The biodistribution of 11C-metformin did not change appreciably during the infusion of unlabelled metformin, and accordingly the estimated effective doses for humans, calculated to 6-7 μSv/MBq, was only approximately 25% higher under metformin infusion compared to baseline (Table S1, supplemental information).

DISCUSSION

11C-metformin has a high affinity for kidney OCTs

Metformin is a hydrophilic organic cation that is not metabolized in humans and it may thus be a good probe drug for organic cation transport (*9,10*). To facilitate examination of the properties of metformin as a drug to study renal OCT2 transport we have developed a novel procedure for radiosynthesis of 11C-metformin. This method gives a higher radiochemical yield in a shorter production time and avoids the use of toxic starting materials compared to a recently published method (*11*) and therefore the present 11C-metformin tracer may have potential applications for human examinations. Since more than 98% of the absorbed dose of metformin is eliminated by the kidneys 11C-metformin

may be a radiopharmaceutical which potentially could provide important information of kidney function both at baseline in normal individuals as well as in patients with kidney disease. The results of the present *in vitro* study showed that 11C-metformin is rapidly transported into the proximal tubule cells and that this transport can be inhibited dose-dependently by cold metformin. Importantly, 11C-metformin is not retained in the proximal tubule cells. Thirty minutes after reincubation of LLC-PK_1 proximal tubule cells previously exposed to 11C-metformin, 40% of the accumulated dose was lost, showing that 11C-metformin is not trapped in the cells. This finding is consistent with previous observations from studies examining the kinetics of cold metformin which demonstrated that more than 90% of the absorbed dose is eliminated by the kidneys (*8,9*). Moreover, the autoradiography studies provided evidence that there is a high cortical uptake with rapid concomitant elimination and accumulation of 11C-metformin in the medullary parts, supporting the view that there is a high renal extraction of 11C-metformin. In the present study, renal extraction efficiency was not measured directly. However, in a subset of pigs we measured the renal clearance of 11C-metformin and 51Cr-EDTA by the constant infusion renal clearance technique. The results of these examinations showed that renal 11C-metformin clearance was approximately 3-4 times higher than the renal clearance of 51Cr-EDTA. This finding is in line

with the results from previous pharmacology studies measuring the renal clearance of metformin in healthy adult Caucasian volunteers and demonstrating a slightly higher renal metformin clearance compared with the renal clearance of 11C-metformin found in 40 kg pigs (*12,13*), and the finding that the renal clearance of metformin is roughly four times higher than the effective glomerular filtration rate (*13*). Furthermore, because of no plasma protein binding and hence free filtration in glomeruli this finding demonstrates that the tubular secretion of metformin during baseline conditions is roughly 75-80 % of the renal metformin clearance. This means that metformin is cleared partially by glomerular filtration (20%) and the remainder by tubular secretion (80%) with no reabsorption. Further, the value of K_1 in the present study, which is of similar magnitude as the plasma flow to the kidneys (*14*), also suggests a high extraction fraction of metformin. We speculate that 11C-metformin may be used to measure the effective renal plasma flow, as the properties of 11C-metformin matches those of p-aminohippurate which has been used as a reference compound for estimation of the effective renal plasma flow (*15*).

In the kidney OCT2 is responsible for basolateral metformin uptake and transport into the proximal tubular cells (*1*). Since OCTs are all uniporters that mediate facilitated diffusion of metformin in either direction (*16*), the luminal

extrusion of metformin into the urine is facilitated by the apical located H+/drug antiporters multidrug and toxin extrusion transporters 1 and 2 (*17*,*18*), elegantly demonstrated using proton pump inhibitors (*19*).

In addition to the organic cation system, renal tubule cells also display a comparable system for organic anions (*20*,*21*). Active transport of organic anions is an essential renal function and organic anion transporters in the proximal tubule are responsible for transport of p-aminohippurate and hippuran analogs (*22*,*23)*. Previous studies have demonstrated drug-drug interaction under conditions where the renal expression of organic anion transporters is reduced upon experimentally induced renal failure (*24*). This indicates that diagnostic information obtained by i.e. 99mTc-MAG3 may be biased under treatment with certain drugs. Interestingly, in OCT1/2 knockout mice, metformin pharmacokinetics but not pharmacodynamics was changed, challenging the presumption that systemic OCT inhibition will affect the effect of metformin (*25*). Even so, 11C-metformin might be a potential indicator for evaluation of the drug interaction of new drug candidates against renal transporters *in vivo* (*26*).

^{11}C-metformin a potential tracer to study kidney function

At the highest dose of metformin tested, the renal handling of 11C-metformin was changed 20% based on SUV value and 25% in peak value in cortex; whereas the liver SUV remained unchanged yet the peak radioactivity occurred much later. This might be explained by metformin having a larger effect on the tissue uptake process than on the elimination transporter proteins, i.e., the capacity of OCT1 (in kidney and liver) and/or OCT2 (in kidney) for metformin is less than that of MATE1/2K viz. plasma membrane monoamine transporter. Since the renal excretion of compounds is affected by urine production and flow, it is also vital to monitor the hydration status of the animals before and during experiments that focus on elimination of compounds from kidneys to bladder.

Cimetidine (*27*,*28*), TBA (*29*), Cisplatin (*30*,*31*) and Quinine (*32*) interacted with metformin either through competition or inhibition of OCTs and/or MATEs, and other drug classes such as tyrosine kinase inhibitors (*33*) or beta-blockers (*34*) are potential substrates for OCT2 and/or MATEs further stressing the importance of developing non-invasive methods to study these interactions. The present study confirms that there is drug-drug interaction, but whether this occurs via OCT or MATE or both needs to be addressed fully.

Biodistribution of ^{11}C-metformin

The high organ specificity of 11C-metformin is in agreement with the known distribution of OCTs (*1*,*35*) and as the present doses of 50-170 MBq 11C-metformin to 40-kg pigs gave high contrast PET-images, it is likely that a dose of e.g. 100 MBq will be sufficient for obtaining similar data quality in human studies. with a radiation burden as little as 0.6 mSv. Conventional gamma-camera renoscintigraphy with 99mTc-MAG3 gives an effective dose equivalent of 1.5 mSv/370 MBq to an adult with normal kidney function provided the person voids at the conclusion of the study and at 4 hour intervals thereafter (*36*). Thus, 11C-metformin PET gives a 0.6 mSv/100 MBq similar to the radiation burden of 99mTc-MAG3, which should favor 11C-metformin PET taking into account voiding and expected superior image quality. Future studies should concentrate on a thorough comparison of properties of 11C-metformin as a radiotracer for quantitative renal PET studies compared with the performance of renal studies acquired with 99mTc-MAG3, 99mTc-DTPA and 131I-hippurate.

CONCLUSION

[*N*-methyl-^{11}C]metformin was prepared by a simple one-step radiosynthesis. This novel tracer undergoes fast renal tubular transport in rat and pig kidneys resulting in high concentrations of un-metabolized 11C-metformin in the urine. Competitive inhibition with unlabelled metformin and other substrates/inhibitors of OCTs indicate that the renal (and hepatic) transport of 11C-metformin takes place via the same transporter proteins. The results of this study therefore indicate that 11C-metformin PET/CT may prove to be useful in humans for characterization and quantification of kidney (and liver) function as well as expression and function of OCTs during normal physiological conditions and in patients with kidney (or liver) diseases.

ACKNOWLEDGMENTS

Preliminary data from this study were presented orally in 2013 at the 20th International Symposium on Radiopharmaceutical Sciences, Jeju, Korea, and in 2014 at the annual meeting of the Society of Nuclear Medicine and Molecular Imaging, St. Louis, Missouri, USA. The study was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK074419) and the Danish Council for Independent Research (Medical Sciences, 09-073658).

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Figure 1. Synthesis scheme for ^{11}C-metformin.

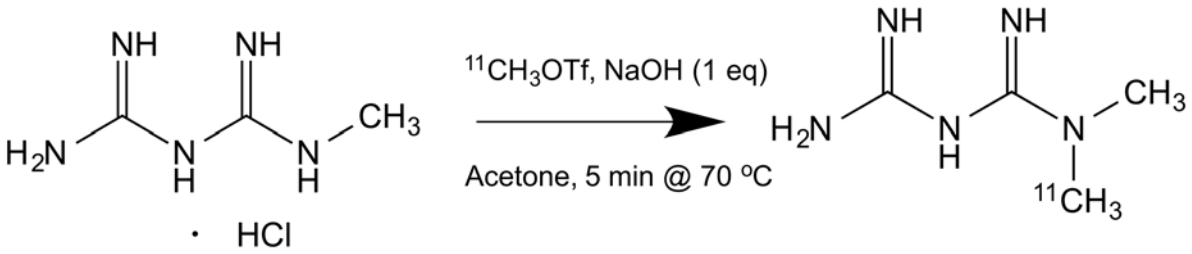

Figure 2. ^{11}C-metformin uptake in OCT1 and OCT2 expressing LLC-PK$_1$ cells (A) at baseline (solid circles) and with increasing concentrations of unlabelled metformin 2,5 mM (solid squares) and 10 mM (solid triangles) (all; n=3). (B) ^{11}C-metformin release kinetics in previously tracer-loaded cells following reincubation in tracer-free medium.

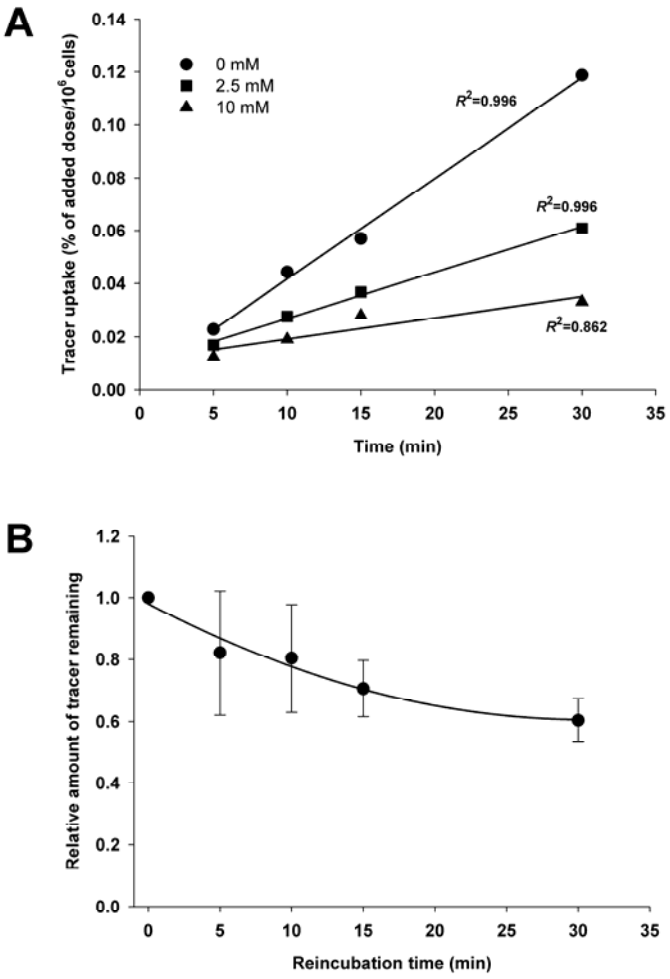

Figure 3. Autoradiography of rat liver (A) and kidney (B) were obtained 2 min after injection of ^{11}C-metformin. There is an augmented uptake of ^{11}C-metformin in the kidney compared to liver and the kidney distribution of ^{11}C-metformin demonstrates that accumulation is most intensive in the renal medulla. *A cutting artifact.

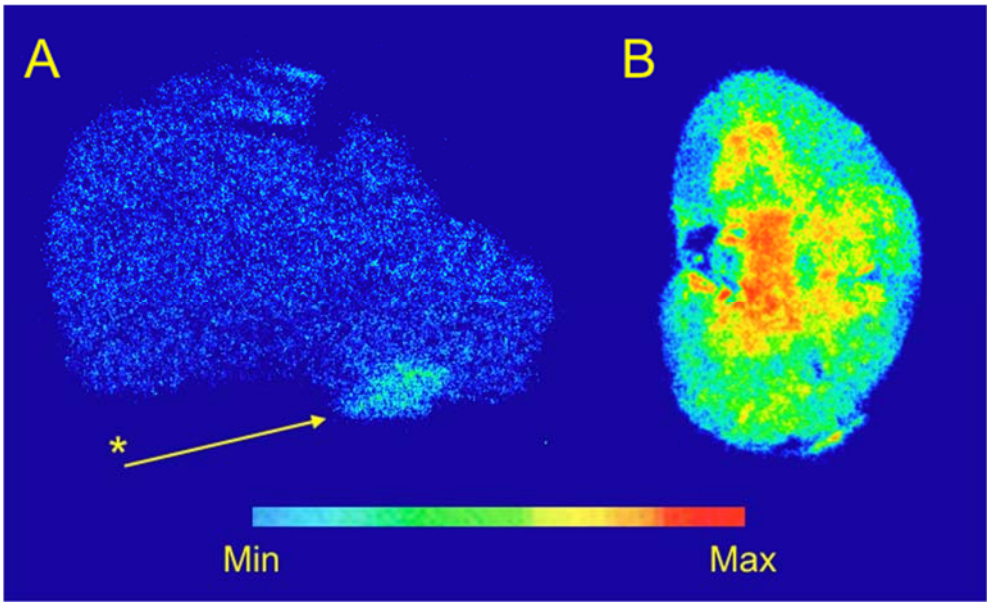

Figure 4. Pharmacokinetics and drug-interaction of ^{11}C-metformin studies in rats subjected to PET. (A) Summed parametric images (I-IV) and corresponding CT image (V). (B) + (C) Representative regional time-activity curves (B) after injection of ^{11}C-metformin show rapid cortical uptake and subsequent accumulation in the renal medulla and pelvis, which yields the 90 minute summed parametric image shown in (C). (D) + (E) Examples of drug-induced changes in renal cortex time-activity curves (D) and the corresponding whole kidney (solid color) and liver (shaded color) SUVs in rats examined at baseline, and after metformin, TBA, cimetidine, cisplatin and quinine (all; n=3) (E).

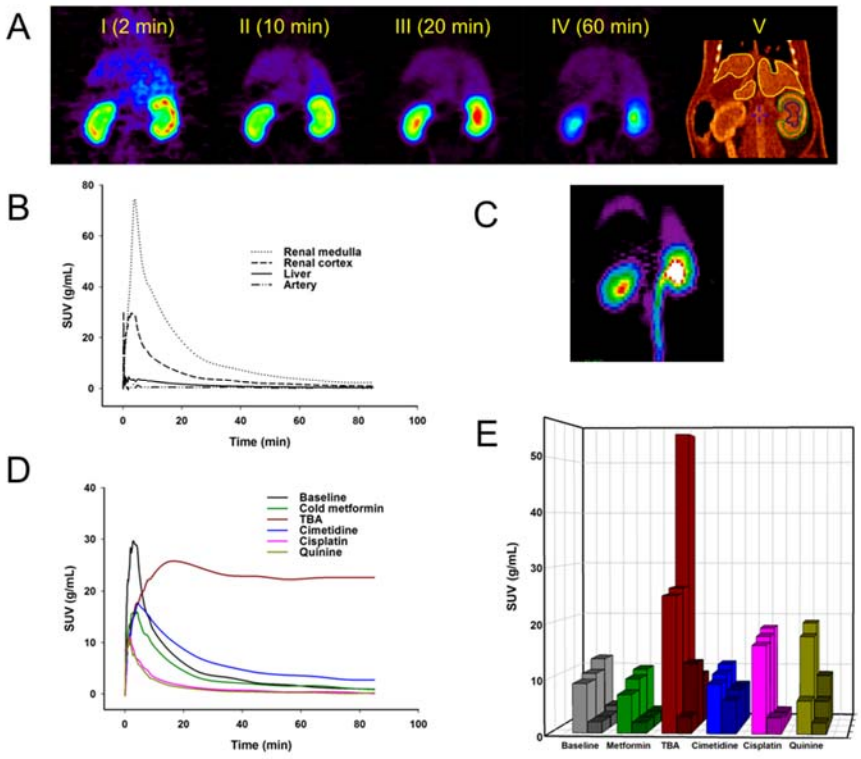

Figure 5. Time-activity curves for renal cortex, liver and blood at baseline (black) and after pretreatment with cold metformin (red). Insert shows ^{11}C-metformin radioactivity in a central coronal plane containing both kidneys at selected times.

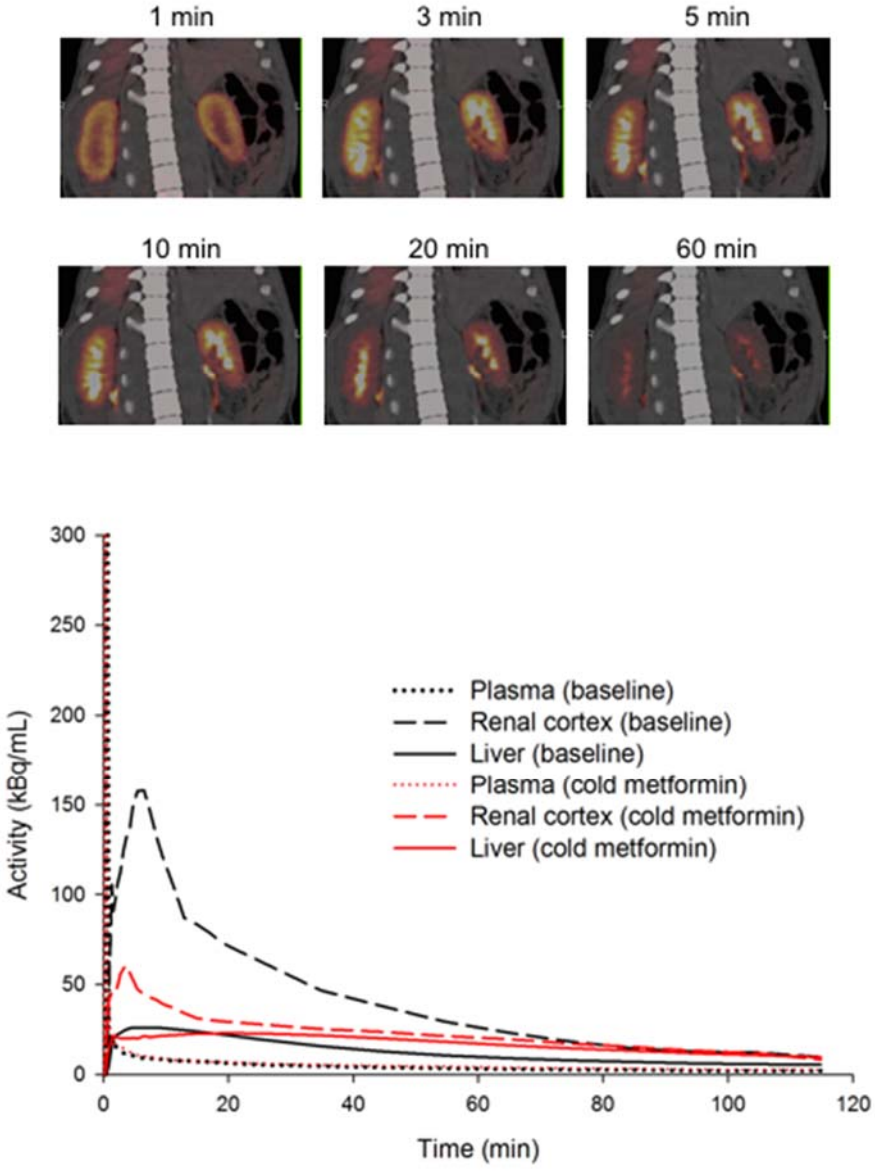

Figure 6. Wholebody pig biodistribution of ^{11}C-metformin.

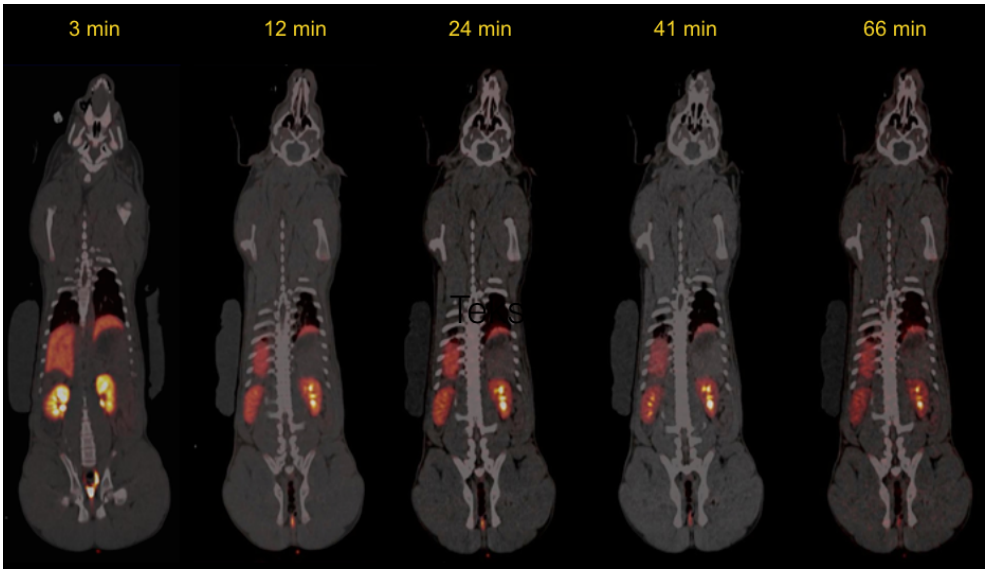

TABLE 1.

Summary of pigs used			
Pig #	**Scan type**	**Purpose**	**biological sample evaluation**
1	Whole body baseline + post metformin	Biodistribution + dosimetri	Plasma + urine metabolite
2	Dynamic PET/CT baseline	pharmacokinetic	plasma metabolite
3	Dynamic PET/CT baseline	pharmacokinetic	plasma metabolite
4	Dynamic PET/CT baseline + post metformin	pharmacokinetic	plasma metabolite
5	Bolus + constant infusion	Renal clearance	Plasma protein binding, plasma + urine metabolite
6	Bolus + constant infusion	Renal clearance	Plasma protein binding, plasma + urine metabolite

TABLE 2. Pharmacokinetics of ^{11}C-metformin before and after competition with unlabelled metformin.

	Baseline	Unlabelled Metformin
K_1 (ml/ml/min)	1.54	0.67
k_2 (min^{-1})	0.22	0.15
Vd (ml per ml plasma)*	7	4.5
$AU_{Ccortex}/AUC_{plasma}$†	6.4	3.9
$Cl_{(plasma \rightarrow urin)}$ (ml/min)‡	382	179

*Distribution volume $Vd=K_1/k_2$.

†$AUC_{cortex}/AUC_{plasma}$ determined at presumed steady state at 60 min post injection.

‡Urinary clearance $Cl=k_2*V_{cortex}*AUC_{cortex}/AUC_{plasma}$; with V_{cortex}=2 x 115 ml.

TABLE 3. Urine clearance of ^{11}C-metformin, ^{51}Cr-EDTA, protein binding and metabolites in plasma from 2 pigs subjected to constant infusion clearance technique experiments.

	Pig no. 5	Pig no. 6
Cl_{MET} (ml/min)	348, 388	334
Cl_{EDTA} (ml/min)	114,117	91
Cl_{MET}/Cl_{EDTA}	3.05, 3.32	3.67
Protein Binding (%)	< 0.5	< 0.5
Metabolites	None	None

Cl_{MET} is the urine clearance of ^{11}C-metformin, Cl_{EDTA} is the urine clearance of ^{51}Cr-EDTA = glomerular filtration rate.