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Measuring the Pharmacokinetic Properties of Drugs with a Novel Surgical Rat Model

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ABSTRACT

Purpose/aim of the study: The pharmacokinetic (PK) parameters in animal models can help optimize novel candidate drugs prior to human trials. However, due to the complexity of pharmacokinetic experiments, their use is limited in academia. We present a novel surgical rat model for investigation of pharmacokinetic parameters and its use in an anti-obesity drug development program. *Materials and methods*: The model uses anesthetized male Wistar rats, a jugular, a femoral catheter, and an insulin pump for peptide infusion. The following pharmacokinetic parameters were measured: metabolic clearance rate (MCR), half-life, and volume of distribution (Vd). Glucagon-like peptide 1 (GLP-1), glucagon (GCG), and exendin-4 (Ex-4) were used to validate the model. The pharmacokinetic parameters of anti-obesity drug candidates X1, X2, and X3 were measured. *Results*: GLP-1 had a significantly higher MCR (83.9 ± 14.1 mL/min/kg) compared to GCG (40.7 ± 14.3 mL/min/kg) and Ex-4 (10.1 ± 2.5 mL/min/kg) ($p < .01$ and $p < .001$ respectively). Ex-4 had a statistically significant longer half-life (35.1 ± 7.4 min) compared to both GCG (3.2 ± 1.7 min) and GLP-1 (1.2 ± 0.4 min) ($p < .01$ for both GCG and GLP-1). Ex-4 had a statistically significant higher volume of distribution (429.7 ± 164.9 mL/kg) compared to both GCG (146.8 ± 49.6 mL/kg) and GLP-1 (149.7 ± 53.5 mL/kg) ($p < .01$ for both GCG and GLP-1). Peptide X3 had a statistically significant longer half-life (21.3 ± 3.5 min) compared to both X1 (3.9 ± 0.4 min) and X2 (16.1 ± 2.8 min) ($p < .001$ for both X1 and X2). *Conclusions*: We present an affordable and easily accessible platform for the measurement of PK parameters of peptides. This novel surgical rat model produces consistent and reproducible results while minimizing animal use.

Keywords: half-life; pharmacokinetics; metabolic clearance rate; jugular vein; femoral vein; drug

INTRODUCTION

The development of high throughput approaches to drug development studies has been driven by the advances in high-speed chemistry and pharmacological screening [1]. There is a constant need for screening more and more compounds as the safety precautions for new drugs and the market competition have increased [2, 3]. Pharmacokinetics involves investigating the mechanism by which a substance is absorbed and distributed within the body, any metabolism of the substance within the body, and finally the effects and the routes of excretion of the substance and its metabolites from the body [4–6].

In the drug development setting, pharmacokinetic parameters (half-life, metabolic clearance rate [MCR], and volume of distribution [Vd]) are important as they allow characterization of the relationship between drug dosage regimens and drug concentration–time profiles [7]. Over the last two decades, the use of pharmacokinetic modeling techniques to aid investigating the drug actions has become a standard practice [8, 9]. Rodents, and in particular rats, are extensively used as experimental models for pharmacokinetic studies,

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helping to optimize and select drugs prior to human trials [8, 10–15].

Drug discovery is extremely expensive, with overall failure rates exceeding 80%, while in the anti-obesity field, the failure rates approach 99% [16–18]. Inadequate pharmacokinetic profiles and poor bioavailability of drug candidates are responsible for 10% of drug failures [19]. Pharmacokinetic experiments incur a significant cost (cost of specialist equipment and consumables, purchasing and maintenance of animals, manpower, and cost of samples' analysis) and these cost implications prevent many groups working on translational research from conducting pharmacokinetic studies during the discovery stages of drug development.

An affordable pharmacokinetic animal model could significantly promote the use of pharmacokinetic early in drug development, and could potentially lead to a higher drug development success rate. As part of an anti-obesity drug discovery program, we have successfully developed a surgical rat model for investigating the pharmacokinetic parameters of candidate drugs. The model includes cannulation of the right internal jugular vein and the left femoral vein, and the use of a widely available and inexpensive insulin pump for intravenous (i.v.) infusion. In this study, we present a surgical rat model for measuring the pharmacokinetic parameters of peptide drugs, its validation, and the results obtained through its use.

MATERIALS AND METHODS

The surgical rat model was used to measure the pharmacokinetic parameters of six peptides. Peptides glucagon-like peptide 1 (GLP-1), glucagon (GCG), and exendin-4 (Ex-4) were used to validate the model. Peptides X1, X2, and X3 were developed and tested as potential anti-obesity drug candidates.

Peptides

GLP-1, GCG, and Ex-4 were purchased from Bachem Ltd. (Merseyside, UK), while peptides X1, X2, and X3 were synthesized by and purchased from Insight Ltd. (Wembley, UK). Peptides were synthesized using an automated fluorenylmethyloxycarbonyl solid phase peptide synthesis method with each amino acid sequentially added from C to N terminus. Peptides were cleaved from the resin and purified using reverse phase preparative high performance liquid chromatography (HPLC) followed by lyophilization. All peptides supplied had a purity of >95% evaluated by HPLC.

Animal Studies

All animal procedures undertaken were approved by the British Home Office under the UK Animal (Scientific Procedures) Act 1986 (Project Licence 70/7236). Adult male Wistar rats (Charles River Ltd., Margate, Kent, UK) were maintained in double-housed cages under controlled temperature (21–23°C) and light-dark cycles (12:12 hr light-dark schedule, lights on at 0700). Animals were allowed ad libitum access to water and ad libitum access to RM1 diet (special diet services) unless otherwise stated. To minimize any non-specific stress effects, animals were regularly handled to allow acclimatization.

Experiments

The study design is shown in Figure 1. Animals were anesthetized with isoflurane, a catheter was inserted in the right jugular vein and a baseline sample was taken. The peptide to be tested (GLP-1, GCG, or Ex-4) was reconstituted at a concentration of 30 nmol/mL; the pump and tubing loaded and primed with the peptide solution prior to catheterizing the left femoral vein. The pump was then connected to femoral catheter and set at a flow rate of 0.3 mL/hr, and ran for 60 min. A single bolus dose of 1.5 nmol was administered only for Ex-4 just before the infusion. Sampling through jugular access was performed throughout the infusion and for 40 min post-infusion. Four animals were used for each peptide pharmacokinetic experiment.

Equipment

The pump used in this study was a Medtronic 407 c insulin pump with a 1.0-mL reservoir (MMT-103A). The catheters used were polyethylene tubing with an internal and outside diameter of 0.46 mm and 0.91 mm respectively (Instech Solomon) [20].

Catheter Preparation

All cannula tubing required was prepared before the start of the procedure. The catheter was cut to 12- and 10-cm length for the jugular and the femoral veins respectively. One end had a 1-mL syringe with a 25-gauge needle attached to it. A 1-mL syringe was filled with heparinized saline (100 IU/mL) and attached to the needle. The other end of the tube was cut with a scalpel blade to create a beveled edge. Catheter implantation was performed with the help of surgical loupes with a magnifying force of 2.5–3.5×.

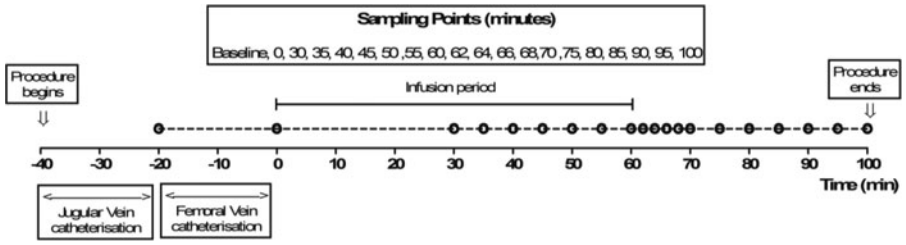

FIGURE 1 Graphical representation of the study design of the surgical rat model in anesthetized male Wistar rats. Plasma sampling points are displayed in black circles over time in minutes.

Pump and Peptide Preparation

Peptides were reconstituted in gelofusine (at a concentration of 30 nmol/mL) after jugular catheter was inserted. Infusion pump was loaded and allowed to run for 20 min to allow the peptide solution to fill femoral catheter. Intravenous infusion lasted for 60 min with an infusion rate of 0.3 mL/hr.

Pump Infusate Concentration Measurement

After the removal of femoral catheter from the femoral vein, the pump was allowed to run inside a microtube for 20 min and the infusate was collected and assayed by radioimmunoassay (RIA). Pump infusate concentration was used to calculate the total amount of peptide infused over 60 min.

Sampling and Hormone Assays

A baseline sample of 500 μL was taken following the insertion of a jugular vein cannulation. A total of 19 other samples were taken at the following time points from the beginning of peptide infusion: 0, 30, 35, 40, 45, 50, 55, 60, 62, 64, 66, 68, 70, 75, 80, 85, 90, 95, and 100 min (Figure 1).

Blood samples were collected in 1.5-mL heparinized microtubes containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA, P2714). The tubes were then spun in a centrifuge (3–18 K SciQuip, Sigma, USA) for 10 min at 3,000 g at 4°C. The plasma supernatant was collected and stored at −80°C until assayed. Measurements of peptides concentration were carried out by established in-house RIA, and all samples were tested in duplicate [21, 22].

Anesthesia

Animals were anesthetized with gas anesthetic (isoflurane via cone mask at 2%) with an appropriate gas scavenging system as described previously [23].

Jugular and Femoral Vein Catheterization

The jugular and femoral vein catheterization was performed as described previously [23].

CALCULATIONS

The pharmacokinetic properties that were investigated and calculated for each of the peptides included half-life, MCR, and volume of distribution. All three peptides were tested at a concentration of 30 nmol/mL at an infusion rate of 0.3 mL/hr. Plasma peptide concentration was measured for 100 min after termination of peptide infusion. Steady state concentration was calculated as the mean of five values obtained at 40, 45, 50, 55, and 60 min of infusion phase. Peptide concentrations were plotted on a semi-logarithmic scale versus time, and computed to yield the slope from which the half-life ($t_{1/2}$) was determined.

Plasma half-life was calculated as follows:

$$t_{1/2}\ (\text{min}) = \ln(2)/\text{gradient},$$

where ln (2) is the natural logarithm of 2 (0.693).

The MCR was calculated using the Tait's formula:

$$\text{MCR (mL kg} - 1\ \text{min} - 1) \equiv \text{infusion rate/steady state plasma concentration.}$$

The Vd was calculated using the following formula:

$$V_d \equiv t_{1/2} \times \text{MCR}/\ln(2),$$

where ln(2) is the natural logarithm of 2 (0.693).

STATISTICAL ANALYSIS

All data are expressed as the mean value ± SD. Comparisons of the pharmacokinetic parameters between the groups were performed using one-way analysis of variation (ANOVA) with Bonferroni's post-hoc test. In

TABLE 1 Comparison between the pharmacokinetic properties (metabolic clearance rate, half-life, and volume of distribution) and the steady-state concentration of glucagon, GLP-1, exendin-4, X1, X2, and X3 peptides as measured in the surgical rat model ($n = 4$)

	Glucagon		GLP-1		Exendin-4		X1		X2		X3	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Metabolic clearance rate (mL/min/kg)	40.7	(14.3)	83.9	(14.1)	10.1	(2.5)	13.9	(8.1)	6.2	(3.5)	5.4	(0.2)
Half-life (min)	3.2	(1.7)	1.2	(0.4)	35.1	(7.4)	3.9	(0.4)	16.1	(2.8)	21.3	(3.5)
Volume of distribution (mL/kg)	146.8	(49.6)	149.7	(53.5)	429.7	(164.9)	81.6	(70.7)	141.9	(43.6)	164.1	(31.3)
Steady state concentration (pmol/mL)	8.4	(4.2)	2.6	(0.4)	16.8	(4.2)	28.7	(13.6)	14.0	(4.2)	24.9	(0.9)

GLP-1: Glucagon-like peptide 1, SD: Standard deviation. Values are shown as mean ± SD.

all cases, values of $p < .05$ were considered statistically significant. The following program was used for statistical analysis: GraphPad Prism, version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

The pharmacokinetic parameters of the peptides are shown in Table 1. The pre-infusion values (baseline levels) of GCG and GLP-1 were approximately 43 and 35 pmol/L respectively. The time-course of the mean plasma concentrations of Ex-4, GLP-1, and GCG are shown in Figure 2, and those of X1, X2, and X3 are shown in Figure 3. Steady-state concentration levels were observed between 30 and 60 min after the start of peptide infusion for all peptides. The steady-state concentration levels of Ex-4, GLP-1, and GCG were 16.8 ± 4.2, 2.6 ± 0.4, and 8.4 ± 4.2 pmol/mL respectively (Table 1). Peptide X1 presented the highest steady-state concentration among all peptides tested in this study (28.7 ± 13.6 pmol/mL).

Glucagon-like peptide 1 had a significantly higher MCR (83.9 ± 14.1 mL/min/kg) compared to GCG (40.7 ± 14.3 mL/min/kg) and Ex-4 (10.1 ± 2.5 mL/min/kg) ($p < .01$ and $<.001$ respectively) (Figure 2). GCG had a significantly higher MCR than Ex-4 ($p < .01$).

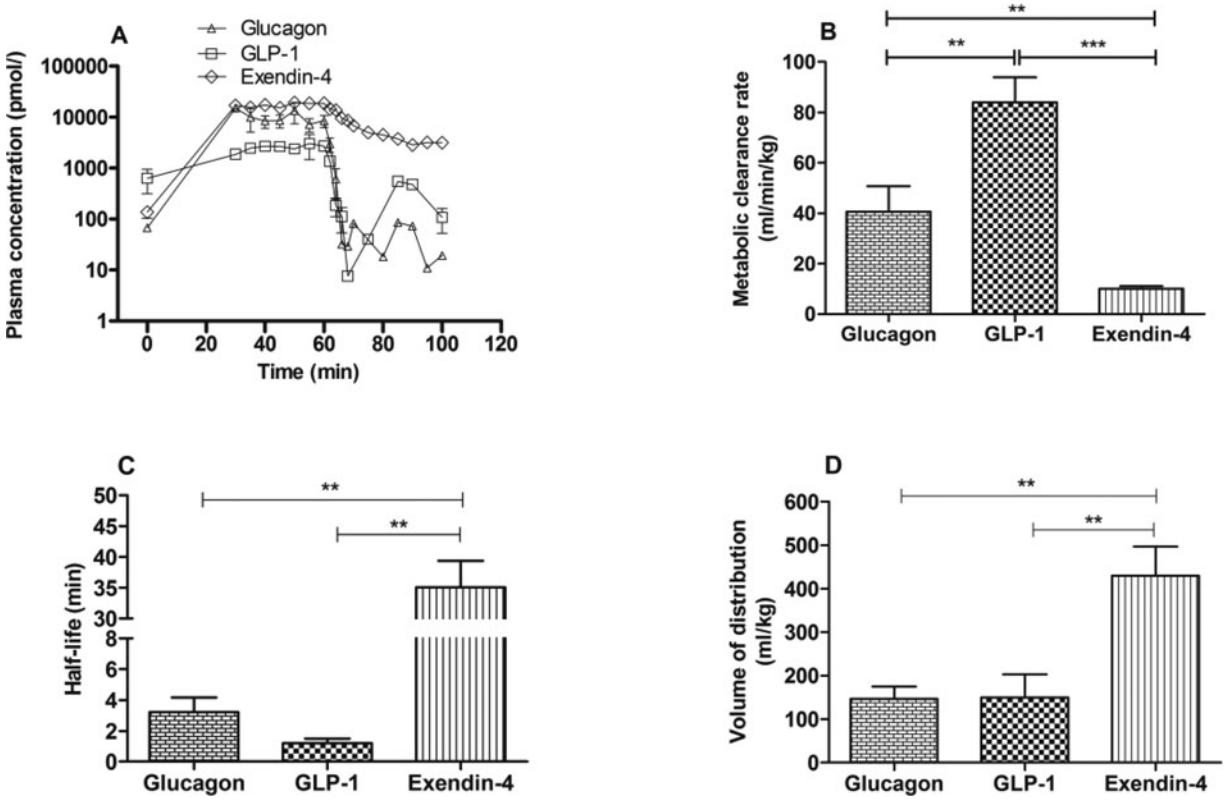

FIGURE 2 Plasma concentrations of glucagon, GLP-1, and exendin-4, and their pharmacokinetic parameters (metabolic clearance rate, half-life, and volume of distribution) using the surgical rat model in anesthetized male Wistar rats. A: semi-logarithmic plasma concentration of peptides over time; B: metabolic clearance rate of peptides; C: half-life of peptides; and D: volume of distribution of peptides. For all experiments, $n = 4$. Statistics with one-way ANOVA. $^{**}p < .01$, $^{***}p < .001$.

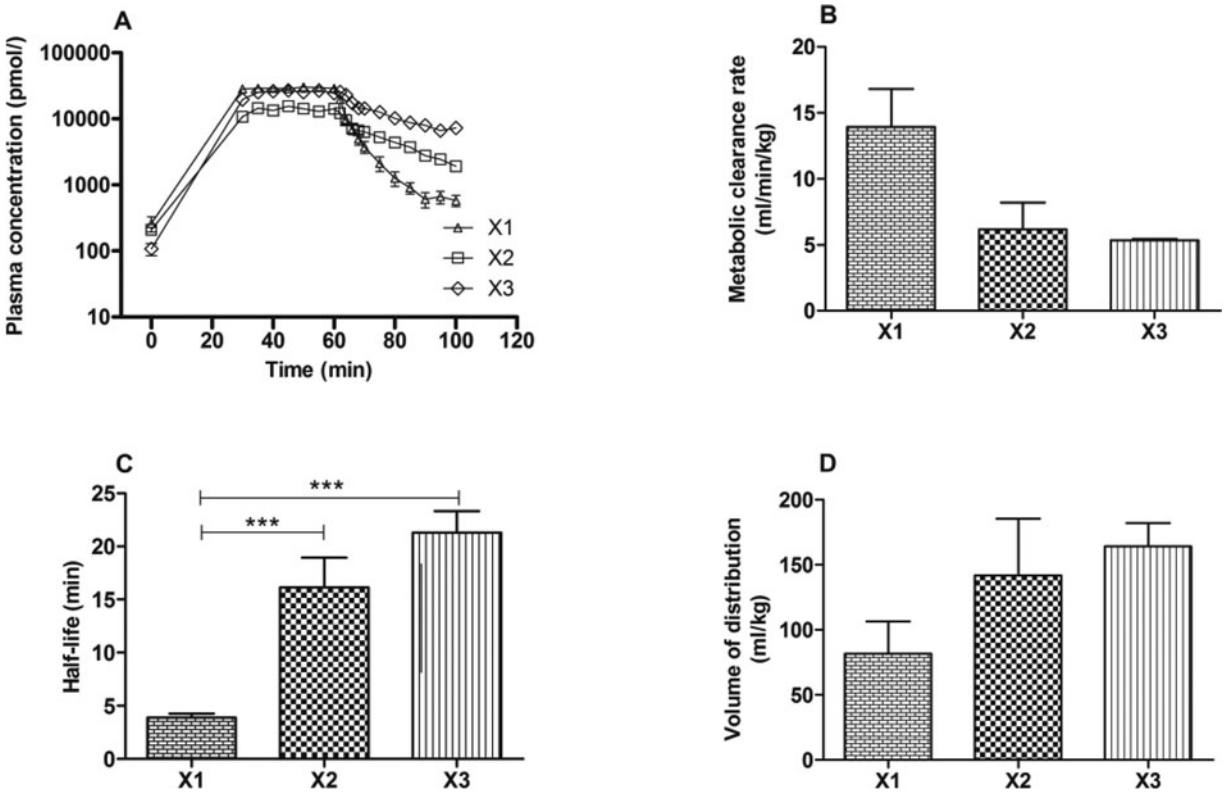

FIGURE 3 Plasma concentrations of peptides X1, X2, and X3, and their pharmacokinetic parameters (metabolic clearance rate, half-life, and volume of distribution) using the surgical rat model in anesthetized male Wistar rats. A: semi-logarithmic plasma concentration of peptides over time; B: metabolic clearance rate of peptides; C: half-life of peptides; and D: volume of distribution of peptides. For all experiments, $n = 4$. Statistics with one-way ANOVA. $^{***}p < .001$.

Exendin-4 had a statistically significant longer half-life (35.1 ± 7.4 min) compared to both GCG (3.2 ± 1.7 min) and GLP-1 (1.2 ± 0.4 min) ($p < .01$ for both GCG and GLP-1).

Exendin-4 had a statistically significant higher volume of distribution (429.7 ± 164.9 mL/kg) compared to both GCG (146.8 ± 49.6 mL/kg) and GLP-1 (149.7 ± 53.5 mL/kg) ($p < .01$ for both GCG and GLP-1).

Peptide X3 had a statistically significant longer half-life (21.3 ± 3.5 min) compared to both X1 (3.9 ± 0.4 min) and X2 (16.1 ± 2.8 min) ($p < .001$ for both X1 and X2) (Figure 3).

DISCUSSION

The surgical rat model presented in this study was developed and used as part of an anti-obesity drug development program in an academic setting. Three different peptides (GLP-1, GCG, and Ex-4) were used to validate the model by measuring their pharmacokinetic parameters and comparing the results with previous published studies. Following results validation, the model was used to measure the pharmacokinetic parameters of three novel anti-obesity drug candidates for humans. The rat model shown here has been used to characterize the pharmacokinetic parameters of more than 50 drug candidates in >200 rats in our experience, producing consistent and reliable results (data not shown due to intellectual property restraints).

The pharmacokinetic properties of drug candidates are important for the designing of new and improved agents, and these can only be calculated by in vivo experiments in animal models. Following validation of our rat model, we measured the pharmacokinetic parameters of drug candidates X1, X2, and X3.

The validation of our model was centered on being able to measure the pharmacokinetic parameters of native peptide hormones. The three peptides used, GCG, GLP-1, and Ex-4, were selected due to the widely different pharmacokinetic parameters that they present and their relevance to the target drug candidate of our anti-obesity drug discovery program.

The pharmacokinetic parameters of GCG, GLP-1, and Ex-4 as reported by this rat model fall well within what has been previously reported [10, 12, 24]. The half-life of GLP-1 reported by Oshima et al. in 1988 [11] (39.5 min) is considered to be outside normal limits; the potential sources of error of that study include the frequent sampling resulting in cardiovascular instability (five samples, 1 mL each, were collected within a period

of 10 min, and after centrifugation the suspension of red cells was reinfused into the animal) [21].

The selection of time-points and volumes of sampling in our paradigm allows for consistent and reproducible measurement of pharmacokinetic parameters while minimizing blood loss and cardiovascular effects. We have previously reported that the total mortality rate in our experience was 0.5% (2/200 rats) [23].

As the field of drug development keeps expanding and the importance of pharmacokinetic studies has become more widely accepted, reports of serial blood sampling and analysis for drug and/or drug metabolites have appeared in the literature with increasing frequency [25]. Regulatory requirements for accurate pharmacokinetic profiles of novel compounds is driving forward the development and refinement of methodology for determining plasma time course data for drugs and/or drug metabolites in rodents.

The development of an animal model for investigating the pharmacokinetic properties of drug candidates in a drug development program is a complex task and requires multiple critical decisions to be made, including the species/strain of animals that is representative of human, the intravenous routes that best serve the pharmacokinetic properties that are investigated, the number and time-points of obtaining the blood samples, the compound dose, the duration/rate of infusion, and the use of a reliable delivery system.

The basic concept underlying the presented surgical rat model is that there are two different intravenous routes required [26, 27]. The first route is used for infusing the drug with the help of a pump, and the second one is required for blood sampling. Previously different routes of intravenous access have been used, the most common routes include the jugular vein, the femoral vein and artery, the saphenous vein, and the carotid artery; each route has its own advantages and disadvantages (Table 2) [10, 11, 15, 25].

In our experience, the use of carotid arteries for sampling requires a higher degree of technical abilities and a longer learning curve. Furthermore, in case of encountering technical difficulties in catheter insertion, blood loss might be higher due to the increased pressure in the arterial system in comparison to the venous one. The saphenous vein presents the advantage of being close to the surface, thus reducing the amount of dissection to locate it; however, its small size makes the catheterization process a challenge.

The femoral vein, in the authors' view, presents some important advantages. It is straightforward to locate, as it is found in a bundle, together with the femoral artery and the femoral nerve. It has an adequate diameter to allow easy insertion of catheters and can be exposed along its course so that more than one venotomies can be attempted if initial attempt fails. Of course, dissecting free the femoral vein from the femoral artery is challenging and great care should be taken not to damage the artery. The use of jugular vein for sampling is one of the preferred methods due to its proximity to the surface and its distance from any other major vessels.

A point worth noting, based on our experience, is that catheters should be secured with the use of more than one ties, in particular the ones used for sampling over the ones used for infusing and whenever multiple samples over a long period of time are to be taken. The use of a single tie to stabilize the catheter has quite often proved inadequate, resulting in the dislodgement of catheter and inability to continue taking samples. One or two extra ties are not time-consuming and provide much more reassurance for uninterrupted blood sampling.

Other equipment routinely used by the authors include reusable cautery pen and magnifying loupes. The use of cautery pen allows for easy and rapid hemostasis, minimizing blood loss, and in experienced hands can also be used for dissection, minimizing operating time. Surgical loupes have proved valuable, especially while climbing the learning curve of femoral vein catheterization but also afterwards, and their magnification is of essence whenever the first catheterization attempt fails and the vessel collapses, making the localization of the venotomy site difficult.

The advantages presented by the use of the proposed animal model for evaluating the pharmacokinetic properties of peptides include the affordability of pump setting (pump and reservoir) due to its low cost, which bypasses the need for an expensive dedicated pump system. The rest of the consumables used are easily accessible and the setting of the procedure can be relatively easily reproduced. At the same time, the design of the study has been optimized to measure the pharmacokinetic properties of gut hormones or analogues developed based on gut hormones. Although our model was optimized for measurement of anti-obesity drug candidates based on gut hormones, it can be used for any type of pharmacokinetic experiments.

A limitation of the current setting is the limited infusion rate that the insulin pump offers. Being originally designed to deliver small quantities of insulin in humans, the Medtronic 407 c pump has a maximum rate of infusion of 0.35 mL/hr. This may pose limitations to researchers who wish a faster infusion rate.

The future research could further validate our model by confirming the presence of the peptide in plasma over different time periods, by using analytical techniques such as mass spectrometry.

In summary, the proposed rat model provides an affordable and easily accessible platform for measuring the pharmacokinetic properties of peptides outside the facilities of large pharmaceutical industries. The authors feel that the surgical rat model for the measurement of the pharmacokinetic parameters of

TABLE 2 Published literature of the pharmacokinetic parameters of exendin-4, glucagon, and GLP-1 in rats

Peptide	Authors/date	Species	Weight (g)	Route	Infusion duration	Dose over time	Total dose given	MCR	Half-life (min)	Vd	(Css) nM
Ex-4	Young et al. (2001)	Male Sprague–Dawley rats	350–370	Infusion: R. Saphenous V. Sampling: R. femoral A	180 min	0.5, 5, and 50 nmol/60 min	1.5, 15, and 150 nmol	0.5 nmol: 8.3 ± 0.7 mL/min 5 nmol: 4.8 ± 0.4 mL/min 50 nmol: 3.7 ± 0.5 mL/min	0.5 nmol: 28 ± 55 nmol: 40 ± 550 nmol: 49 ± 7	—	0.5 nmol: 1.1 ± 0.15 nmol: 19 ± 1. 950 nmol: 262 ± 60
GCG	Emmanouel et al. (1978)	Male Sprague–Dawley rats	250–350	Infusion: Jugular V. Sampling: Carotid A.	45 min	0.03–0.13 nmol/45 min*	0.03–0.13 nmol	31.8 ± 1.2 mL/min/kg	—	—	—
	Kervran et al. (1990)	Male Wistar rats	250–280	Infusion: Saphenous V. Sampling: Carotid	45 min	0.56 nmol/45 min	0.56 nmol	36 ± 5 mL/min/kg	1.9 ± 0.1	100 ± 10 mL/kg	—
	Oshima et al. (1988)	Male Wistar rats	500–550	Infusion: Femoral V. Sampling: Jugular V.	60 min	0.57 nmol/60 min	0.57 nmol	46.7 ± 13.3 mL/min/kg	5.8 ± 1.0	240 ± 70 mL	—
GLP-1	Young et al. (2001)	Male Sprague–Dawley rats	350–370	Infusion: R. Saphenous V. Sampling: R. Femoral A.	180 min	0.5, 5, and 50 nmol/60 min	1.5, 15, and 150 nmol	0.5 nmol: 35 ± 11 mL/min 5 nmol: 34 ± 4 mL/min 50 nmol: 38 ± 3 mL/min	0.5 nmol: 1.2 ± 0.95 nmol: 0.9 ± 0.650 nmol: 0.5 ± 0.2	—	0.5 nmol: 0.32 ± 0.065 nmol: 2.49 ± 0.4550 nmol: 20.53 ± 2.94
	Oshima et al. (1988)	Male Wistar rats	500–550	Infusion: Femoral V. Sampling: Jugular v.	60 min	30.3 nmol/60 min	30.3 nmol	18.6 ± 8.6 mL/min/kg	39.5 ± 15.5	500 ± 100 mL	—

Ex-4: exendin-4, GCG: glucagon, GLP-1: glucagon-like peptide 1, Css: steady-state concentration.
– signifies that the respective parameter was not reported.
*Animals also received a priming dose of 0.3–1.3 nmol of glucagon at the beginning of the experiment.

peptides described in this study can be a useful tool that can produce consistent and reproducible results while minimizing animal use.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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AUTHORS CONTRIBUTIONS

Ioannis Christakis, James Minnion, Rebecca Scott, and Joyceline Cuenco carried out the design and coordinated the study, participated in all of the experiments, and prepared the manuscript. Stephen Bloom, Fausto Palazzo, and Tricia Tan provided assistance in the design of the study, coordinated most of the experiments, and participated in manuscript preparation. All authors have read and approved the content of the manuscript.

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