An Isolated In-Situ Rat Head Perfusion Model for Pharmacokinetic Studies

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INTRODUCTION

The effects of drugs on tissues in the head have mainly been limited to influx studies in the brain (1,2). Sakane’s group has recently explored the efflux of solutes from the brain (3). The estimation of distribution pharmacokinetic parameters in a given body region such as the head is generally difficult due to recirculation effects. The most important organ in the head is the brain, which is selectively permeable to blood-borne substances through the capillary endothelium (BBB). Techniques used to study the transport of solutes across the BBB have included indicator dilution (4), brain uptake index (1), internal carotid artery and secured by two ligatures. Immediately after arterial cannulation, blood flowed through the cannula.


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ABBREVIATIONS: BSA, bovine serum albumin; BBB, blood-brain barrier; RBC, red blood cells; PET, positron emission tomography.

Few studies have attempted to examine the efflux parameters of solutes in the head. Of these few studies, outflow perfusate sample collections lasted for less than one minute (3,11). An underestimation of the tail of the outflow curve as a consequence may cause difficulties in accurately defining statistical moments and in modelling. We have used non-linear regression analysis, incorporating capillary permeability, tissue diffusion and vascular heterogeneity in conducting a number of pharmacokinetic studies using the single pass perfused liver and hind limb preparations both in rats (12–16) and in man (17).

The purpose of this study was to develop a single pass rat head perfusion model (including the perfusion of the right side of the brain) viable for at least one hour to allow for pharmacokinetic studies. The model has an extended viability, facilitated by (a) the introduction of a continuously oxygenated perfusate; (b) the ligation of various neck vessels supplying the contralateral side of the brain so that recirculation and the mixing of systemic blood with the oxygenated perfusate could be prevented; (c) maintaining the rat brain preparation at 37 ± 2°C; (d) monitoring systolic pressure, oxygen consumption, pH, wet/dry brain tissue weight ratio and enzyme release. The multiple indicator dilution technique and statistical moments estimation with the double inverse Gaussian distribution applied previously to other organ pharmacokinetic parameter estimation (18,19), was also used in this study.

MATERIALS AND METHODS

Surgical Procedure

Female Sprague-Dawley rats weighing 230–340 g (n = 15) were fed a standard commercial diet and water ad lib. The experiments conducted in this study adhered to the “Principles of Laboratory Animal Care” and were given ethical approval by the University of Queensland Animal Ethics Committee. The rats were anaesthetised with a single i.p. injection of ketamine (8 mg/kg; Parnell Laboratories, NSW, Australia) and xylazine (10 mg/kg; Bayer, NSW, Australia). Prior to arterial cannulation, heparin sodium (500 IU/kg, DBL, Australia) was injected into the femoral vein using a 27G × 1/2” needle U-100 insulin syringe (Terumo, Japan). Arterial cannulation involved the isolation of the right carotid artery followed by the ligation of the pterygopalatine and external carotid arteries using 6-0 surgical silk (Ethicon, Australia). A 22G × 1” i.v. catheter (Terumo, Japan) was inserted into the carotid artery, advanced into the internal carotid artery and secured by two ligatures. Immediately after arterial cannulation, blood flowed through the cannula.

Perfusion System

The perfusion was started as soon as possible through the arterial cannula with a peristaltic pump (Masterflex L/S standard drive system, Cole-Parmer, IL). The perfusion fluid consisted of 3-[N-Morpholino]propane-sulphonic acid (MOPS)-buffered Ringer’s solution with a final composition of (in g/L): NaCl 6.9, KCl 0.35, KH₂PO₄ 0.16, MgSO₄·7H₂O 0.29, CaCl₂·2H₂O 0.24, MOPS 5.23 and glucose 2. BSA (Sigma, USA) (2 or 4% w/v) was added followed by adjustment of the pH to 7.4 using HCl/NaOH. The buffer was filtered (no.541, Whatman, England) prior to use.
Initial perfusate conditions consisting of 37°C with 2% BSA were varied in order to confirm that the experiments conducted at 37°C and with 2% BSA were optimal. Variations in conditions included a lower temperature (30°C), the presence of 10% RBC in the perfusate to increase the amount of oxygen available (refer below for preparation) and higher and lower percentages of BSA (4% and 0%) in the perfusate. A flow rate of 4.6 ml/min was used (3). To enable venous cannulation, the right parotid gland was excised to expose the external jugular vein. A ligature was placed around the anterior facial vein followed by the insertion of a 20G × 1 1/4″ i.v. catheter (Terumo, Japan) into the posterior facial vein and secured with another ligature. A mixture of perfusate and blood flowed through the cannula. The superior sagittal sinus was not cannulated in this method due to the small size of the rat and the fact that the posterior facial vein was more accessible and located on the same side of the rat as the arterial catheter. Following cannulation, the left carotid artery, external and internal jugular veins were ligated to prevent recirculation and mixing of systemic blood with the perfusate. After the commencement of the perfusion, the rat was euthanized by a cardiac injection of concentrated potassium chloride.

**Preparation of Blood for Perfusion**

Greyhound (dog) RBC used in perfusion experiments were obtained from the University of Queensland Veterinary School during routine collections for clinical studies. Dog erythrocytes have been included in the buffer of previous perfusion studies to increase the amount of oxygen available for consumption (8,20). The blood was collected into commercial acid citrate dextrose solution and used within 24 hours of collection. The blood was centrifuged at 2500 rpm (400 × g) for 20 minutes (Beckman Instruments, Palo Alto, California, USA). The plasma and white cell layer were removed. The cells were then washed twice with cold sterile saline and centrifuged at the same speed for 10 minutes. The final wash was with cold MOPS buffer followed by resuspension of the RBC in the buffer and kept at 4°C overnight. On the day of the experiment, the suspension was centrifuged at 2500 rpm for 20 minutes and the supernatant removed. 100ml of the RBC were then added to 900ml of MOPS buffer for a final composition of 10% RBC in MOPS buffer.

**Impulse-Response Studies**

After a 20 minute stabilisation period following the start of the perfusion, 20 µl of the injectate including [3H]-water (740 mBq/ml, University of Queensland, Australia) (water space marker), [14C]-sucrose (250mCi/2.5ml, DuPont, Boston, Massachusetts, USA) and [99mTc]-RBC (~183mBq/ml, Royal Brisbane Hospital, Queensland, Australia; RBC were labelled with 99mTechnetium using an UltraTag® RBC Kit, Mallinkrodt Medical, USA) (vascular marker) and perfusate were injected as a rapid bolus via the in-flow cannula into the carotid artery. Outflow perfusate samples were collected immediately following injection in a fraction collector over 28 minutes at 1 second each (samples 1 to 20), 2.5 seconds each (samples 21 to 40), 4 seconds each (samples 41 to 70), 10 seconds each (samples 71 to 76), 30 seconds each (samples 77 to 84), 1 minute each (samples 85 to 89) and 5 minutes each (samples 90 to 92). An aliquot from each outflow sample (20µl) containing [3H]-water, [14C]-sucrose and [99mTc]-RBC was transferred to Eppendorf tubes for counting in a Cobra II™ Auto-Gamma® counter (Packard Instrument Co., USA). The extent of technetium decay during the process of counting was accounted for in subsequent analysis by the following equation:

$$C_t = C_0 e^{-kt}$$  \hspace{1cm} (1)

where $C_t$ is the number of counts in a particular sample at time $t$ (min) and $k$ is the first order rate constant for $[99mTc]$ decay. After allowing the technetium in the samples to decay for at least 3 days, a further aliquot from each outflow sample (20 µl) was transferred to scintillation vials containing 5 ml UltimaGold™ scintillation fluid (Packard Instrument Co., USA) for beta counting in a Minaxi β Tri-Carb® 4000 series liquid scintillation counter (Packard, USA). Quenching was accounted for automatically by a built-in microprocessor after measuring the activity of an external radon source. The number of counts obtained from both β and γ samples were compared with the counts of the standards. Concentrations of [3H]-water, [14C]-sucrose and [99mTc]-RBC in each sample were expressed as the fraction of the dose injection per millilitre of perfusate.

**Morphological Studies**

**Vascular Casting**

Following surgery for an in-situ experiment and the stabilisation of systolic pressure (to approximately 70 mm Hg), 5mg/ml solution of Mercox (Mercox-Jap, Vilene Co., Tokyo, Japan) casting resin was made up with its catalyst and injected manually through the injection port of the arterial catheter in 3 rats. As much of the 5ml solution was injected up into the internal carotid artery to the right side of the brain as possible while maintaining the pressure at approximately 70 mm Hg. Following the injection, the entire rat was gently submerged in hot water to set the resin and then left overnight at room temperature. The head of the rat was then removed and placed in 5M sodium hydroxide for a few days to remove bone, skin and muscle from the resulting cast.

**Fluoroscopic Imaging**

Following the surgery for a typical in-situ experiment and the stabilisation of the systolic perfusion pressure (as mentioned above), a rat was placed under a fluoroscopy unit with image intensifier (Super 80CP, Phillips, Netherlands) and an Omnicaque solution (Sterling Pharmaceuticals, NSW) was perfused at a rate of 4.6 ml/min through the right internal carotid artery. This was continued until the medium came out of the venous catheter.

**Biochemical Studies**

Inflowing and outflowing perfusate samples were analysed for pO2, pH (1312 blood gas manager, Instrumentation Laboratory, USA) and enzyme release (creatine kinase and lactate dehydrogenase) using a 747 Autoanalyser (Hitachi, Japan) after each injection. Cerebral oxygen consumption (µmol/min/g brain) was determined by the formula: