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Understanding the Impact of Critical Illness on Drug Pharmacokinetics - Scientifically Robust Study Design

Francia Van der Merwe[1], Steven Wallis[2] and Andrew Udy[1,2]*

[1]*Department of Intensive Care Medicine, Royal Brisbane and Womens Hospital, Herston, Queensland, Australia*
[2]*Burns, Trauma, and Critical Care Research Center, University of Queensland, Royal Brisbane and Womens Hospital, Herston, Queensland, Australia*

Abstract

Critical illness causes profound pathophysiological changes in almost all organ function, particularly the cardiovascular, respiratory, renal and hepato-billiary systems. These changes can alter the pharmacokinetic parameters of many commonly prescribed medications, such that sub-therapeutic or toxic drug levels are very real possibilities, particularly when employing standard drug dosing regimes. Furthermore, adequate drug exposure, especially when prescribing antimicrobial therapy, is often essential in minimizing morbidity and mortality in this setting. As such, some consideration of the unique characteristics of this patient population are essential when planning future pharmacological studies in this area.

The primary aim of this review is to examine the main pathophysiological changes seen in critical illness, particularly those encountered with sepsis, and explore the impact of these changes on drug pharmacokinetics. Secondarily, we also review some of the key methods available to investigate altered organ function and pharmacokinetics in this setting, with a focus on newer novel technologies.

Introduction

The pathophysiological changes associated with critical illness, especially those observed with the systemic inflammatory response syndrome (SIRS) and sepsis have been well described [1]. It is becoming increasingly apparent that such changes will result in significantly altered pharmacokinetics (PK) for both enterally and parenterally administered medications in this setting [2-4]. How this alters clinical outcomes is still a matter of study, although it is not difficult to envisage how such changes may cause either toxic or sub-therapeutic drug concentrations, the later potentially resulting in treatment failure. Of key interest to clinicians is the potential effects on antibiotic PK, given the established relationship between early and appropriate antimicrobial therapy, and improved mortality in septic patients [5,6]. Furthermore, optimal dosing of these agents is essential to minimizing the development of antimicrobial resistance [7], an increasingly difficult problem in modern medical practice.

Some medications used in the intensive care setting, such as vasopressors and sedatives, can be titrated to specific clinical endpoints. However, the clinical effect of antimicrobial therapy is often not immediately apparent. Although it is common practice to measure plasma drug levels of certain antibiotics (such as aminoglycosides) in an attempt to prevent complications of toxicity [8], such practices are not routinely performed for all agents. A further concern is that plasma levels of drugs may not reflect adequate tissue or target site penetration, providing a falsely re-assuring sense of efficacy.

In this respect, an improved understanding of the pathophysiological changes associated with critical illness is crucial in guiding the appropriate and safe administration of medications in the intensive care unit (ICU). The primary purpose of this article is to review the pathophysiological changes seen in the critically ill, with particular reference to those associated with sepsis, and how this may influence PK properties. Secondarily, we will review some of the key methods available to quantify such changes in the individual patient, with a view to informing future pharmacological or toxicological study design. Given the clinical imperative to dose antibiotics correctly, this group of drugs will be used to illustrate key point's through-out the paper.

Basic Pharmacokinetic Principles

Pharmacokinetics refers to the changes in drug concentration in the body over time. Key PK characteristics for any agent include:

Volume of distribution (V_d)

Volume of Distribution (V_d) is the apparent volume of fluid into which a drug disperses and is reflected by the measured plasma concentration. The physicochemical properties of a drug which account for this dispersion includes its affinity for water (hydrophilic drugs) or lipids (lipophilic drugs), the degree of protein binding and its molecular size and charge.

Clearance (CL)

Clearance (CL) is defined as the volume of plasma that is effectively cleared of a drug per unit time. Total clearance is the sum of the individual clearances for each organ system or tissue that is involved in the metabolism and/or elimination of a particular drug.

The renal clearance of a drug depends on its degree of filtration at the glomerulus and whether it undergoes active tubular secretion or re-absorption. The glomerulus allows small, non-protein bound and hydrophilic drugs to be filtered, whereas highly protein bound drugs and large molecules are reflected by the glomerular membrane. The

***Corresponding author:** Andrew A Udy, Staff Specialist, Department of Intensive Care Medicine, Royal Brisbane and Womens Hospital, and Senior Lecturer, Burns, Trauma and Critical Care Research Center, University of Queensland, Herston, Queensland, Australia, Tel: +61736368111; E-mail: andrew_udy@health.qld.gov.au

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renal tubule can excrete drugs through active secretary mechanisms and/or reabsorb drugs from the tubular ultra-filtrate.

Hepatic metabolism of drugs involves staged enzymatic reactions which function to increase the solubility of molecules, enabling their excretion in urine or bile. Liver enzyme systems such as the cytochrome P450 system play an integral role in this process.

The lung may be involved in the clearance of volatile compound such as anaesthetic agents and ethanol. Here the rate of clearance will be determined by the cardiac output, the diffusion capacity of the drug and the minute ventilation.

Plasma half life (t ½)

Plasma half life (t ½) is defined as the time required to decrease the plasma concentration by one half.

Protein binding

Highly protein bound drugs typically have a small V_d. The degree of protein binding can be affected by various biochemical and physiological changes that occur in critical illness [9]. Decreased protein binding occurs with alterations in systemic pH, the co-administration of highly protein bound drugs and with conditions associated with hypoalbuminaemia such as chronic liver disease. Conditions associated with increased levels of endogenous molecules with a high affinity to albumin such as uremia [10], hyperbilirinaemia [11], and increased free fatty acid levels [12], seen in sepsis and with parenteral nutrition, can alter drug protein binding. Decreased protein binding of usually highly bound drugs, leads to an increase in the unbound fraction of the drug which is associated with an increase in V_d [13] and CL of the drug [9, 14].

Hydrophilic drugs are largely limited to the extracellular space and demonstrate poor tissue penetration. They typically have a small V_d, and are predominantly renally cleared. By contrast, lipophilic drugs have a higher V_d with good tissue penetration. These agents are predominantly hepatically eliminated [3].

Altered Organ Function and Pharmacokinetics in the Critically Ill

Sepsis is a common reason for admission to the ICU. The incidence of sepsis is increasing in the United States and is listed as the 10th leading cause of death [15]. In Australia and New Zealand the incidence of severe sepsis in adults admitted to ICU is as high as 0.77 per 1000 population, with an ICU mortality of 26.5% and a 28 day mortality of 32.4% [16].

The systemic inflammatory response syndrome (SIRS) is characterized by a collection of clinical and biochemical features [17], often observed in response to tissue damage, and associated with the release of interleukins, cytokines and other mediators of inflammation [18]. SIRS can be encountered with both infectious and noninfectious etiologies, and in the presence of a suspected or proven infection, is then referred to as 'sepsis' [17]. The development of organ dysfunction and hypotension refractory to fluid resuscitation in a patient with sepsis is termed septic shock [19]. Table 1 summarizes the changes in organ function, and resultant PK, commonly seen in patients manifesting SIRS or sepsis.

The cardiovascular system

Cardiac output: The cardiovascular manifestations of sepsis are well documented [20,21]. The classically described hyperdynamic shock syndrome, caused by a low systemic vascular resistance and a high cardiac output [21], is commonly seen in patients with severe sepsis and septic shock. Intravenous fluid loading and the introduction of vasoactive agents if hypotension persists are key initial therapies in the treatment of shock [22,23]. These resuscitative measures may further augment this hyperdynamic state, resulting in supra-normal cardiac indices, and enhanced major organ blood flow [24,25].

Conversely, direct myocardial depression is also a recognized complication of septic shock [26]. Unappreciated it can result in impaired organ perfusion [20], and may contribute to the multiple organ dysfunction syndrome (MODS), a frequent sequelae of untreated septic shock [27]. In terms of PK considerations, decreased organ perfusion can result in renal and/or hepatic dysfunction and decreased drug CL and metabolism. This may lead to prolonged drug elimination half-lives, increased drug concentrations and the accumulation of active or toxic metabolites [28].

The microcirculation: Altered microvascular blood flow in sepsis has been confirmed in multiple studies [29-31]. The microcirculation is defined as a network of blood vessels with a diameter less than 300μm. It functions to enable the transport of nutrients and oxygen to the tissues and is a conduit for the delivery of therapeutic drugs to target tissues. The microcirculation's regulatory mechanisms are disrupted in sepsis, causing absent or reduced flow in some capillaries and abnormally high flow in others [32]. It has been postulated that this may account for the cellular dysoxia and abnormal tissue oxygen extraction demonstrable in sepsis [33].

A PK consequence of microcirculatory dysfunction is likely to include reduced antimicrobial tissue penetration in patients with sepsis [34]. Importantly, these sites are often the foci of infection [35], and multiple authors have confirmed low tissue antibiotic concentrations in patients with septic shock [36-38]. Of great concern to the clinician, is that decreased antimicrobial penetration into tissue will result in treatment failure, or the emergence of antibiotic resistance through partial treatment.

The capillary leak syndrome: The endothelial integrity of the capillaries can be damaged by various endogenous inflammatory mediators [39]. These inflammatory mediators which include interleukins, kinins and cytokines, are produced in response to endotoxins released from various infectious agents [40,41]. Endothelial damage increases capillary permeability [41] and is often seen in sepsis [42,43]. Increased capillary permeability in combination with fluid resuscitation, will cause significant fluid shifts from the intravascular to the interstitial space [44,45], a process known as "third spacing".

Plasma proteins such as albumin, are also lost into the interstitium during this process, leading to increased interstitial binding of highly protein bound medications. Joukhadar and colleagues have confirmed such a consideration by demonstrating decreased interstitial concentrations of unbound Cefpirome in septic patients, when compared to healthy controls [34].

Third spacing increases the V_d of hydrophilic drugs with a subsequent decrease in plasma drug concentrations [46, 47]. In contrast, the V_d of lipophilic drugs is unlikely to be affected by third spacing [3]. Cardiac failure and renal failure can both cause further edema and fluid retention in the critically ill, increasing the V_d of hydrophilic medications [4].

The respiratory system

Sepsis is implicated in 25%-40% of cases with Acute Respiratory

System	Physiological Disturbance	Altered Pharmacokinetics
Cardiovascular	Hyperdynamic circulation - ↑ CO and organ blood flow - ↑ with fluids & vasoactive medications	↑ CL Sub-therapeutic concentrations
	Myocardial depression - ↓ CO & organ perfusion	↓ CL Drug accumulation / toxicity
	Microciruclatory failure	↓ Target tissue penetration
	Endothelial dysfunction & Capillary leak	↑ V_d (hydrophilic drugs)
Respiratory	↓ gas exchange - Hypoxemia & hypercapnoea	↓ RBF ↓ Renal CL
	Endotracheal intubation	Impaired aerosol delivery
	Positive Pressure Ventilation	↓ CL ↑ V_d (hydrophilic drugs)
Renal	Augmented Renal Clearance - ↑ GFR +/- tubular function	↑ CL Sub-therapeutic concentrations
	Acute Kidney Injury - ↓ GFR +/- tubular function	↓ CL Drug accumulation / toxicity ↑ V_d (hydrophilic drugs) Altered protein binding (uraemia)
	Renal Replacement Therapy	↓ CL Drug accumulation / toxicity
Hepatobilliary	Hepatic dysfunction - ↓ HBF - altered enzyme (CYP450) function	↓ CL Drug accumulation / toxicity
	Acute phase response - ↑ production AAG	Altered protein binding (Basic drugs)
	Hypoalbuminaemia	↑ Free fraction ↑ CL (highly protein bound drugs)
Other	Gastrointestinal function - ↓ motility	↓ drug absorption Sub-therapeutic concentrations
	Integument - ↓ perfusion	↓ drug absorption Sub-therapeutic concentrations
	Polypharmacy	Enzyme inhibition / induction Direct competition (protein binding) Variable CL

AAG - α1-Acid Glycoprotein; CL – Clearance; CO – Cardiac Output; GFR - Glomerular Filtration Rate; HBF - Hepatic Blood Flow; RBF - Renal Blood Flow; V_d - Volume of Distribution

Table 1: Changes in Organ Function and Drug Pharmacokinetics in the Critically Ill.

Distress Syndrome (ARDS), the focus of which may be either a primary pulmonary or an extra pulmonary infection [48]. Local or systemic insults cause severe pulmonary inflammation and diffuse damage to the alveolar-capillary membrane, resulting in impaired gas exchange, and type I or type II acute respiratory failure [49].

Severe refractory hypoxemia (PaO2<40mmHg) can cause functional renal insufficiency due to renal artery vasoconstriction, with renal hypoperfusion and an impaired GFR [50]. Lung protective ventilation strategies, commonly used in patients with ARDS, include ventilation with small tidal volumes, and permissive hypercapnoea [51]. Hypercapnoea can reduce renal blood flow (RBF) directly by causing renal vasoconstriction [52], or indirectly by causing systemic vasodilatation, with the subsequent release of noradrenaline and the stimulation of the renin-angiotensin-aldosterone system which will lead to a decrease in RBF [50]. These changes may lead to decrease clearance and increased plasma levels of renally excreted drugs.

Positive pressure ventilation (PPV): Various researchers have demonstrated that mechanical ventilation is responsible for altered pharmacokinetics of many commonly prescribed antibiotics [53-55]. Positive pressure ventilation, with or without positive end-expiratory pressure (PEEP), is associated with significant haemodynamic effects. PPV and PEEP generate increased intra-thoracic pressures which decrease the venous return to the heart, causing a fall in cardiac output [56]. PPV is also associated with decreased hepatic and renal blood flow.

The reduction in renal blood flow translates into a decreased GFR and urine production [57,58]. Although controversial [50], data suggests that PEEP can alter renal neurohormonal factors, leading to an increase in both plasma renin and aldosterone activity, resulting in reduced RBF and GFR. The application of PEEP is associated with increased antidiuretic hormone secretion with associated fluid retention and the formation of tissue oedema [59]. A clinical consequence of this

is an increase in the V_d of hydrophilic medications with resultant sub-therapeutic concentrations. The hepatic elimination of drugs with a high hepatic-extraction ratio may also be reduced during mechanical ventilation due to PPV [57,60].

Administration of medications via the endotracheal tube is well established. Aerosol deposition in the lower respiratory tract during mechanical ventilation is influenced by numerous factors. These may include the mode and settings of ventilation, the heat and humidification of the inspired gas, the endotracheal tube size and the method by which the Metered Dose Inhaler (MDI) is connected to the ventilator circuit [61,62]. In ventilated patients with pneumonia, aerosol antimicrobial administration may achieve high antibiotic concentrations at the target site, with the possibility of low systemic drug absorption. However, large amounts of drug particles are deposited in the anatomical dead space without reaching the alveolar compartments, resulting in lower than expected target site drug concentrations [63]. Newer pulmonary drug delivery systems may achieve higher drug concentrations in the alveolar tissues [63].

The renal system

Augmented renal clearance (ARC): Recently, investigators [64-66] have defined this phenomenon as the enhanced renal elimination of circulating solute, principally due to elevated glomerular filtration. As expected, this can have potentially profound effects on antimicrobial PK in the critically ill [66]. ARC is especially important in sepsis, where enhanced antibiotic CL can lead to sub-therapeutic dosing and potentially treatment failure [67,68]. As previously discussed, SIRS/Sepsis and the associated hyperdynamic circulation can lead to an increased cardiac output. In experimental animal studies, increased cardiac output correlates with an increase in renal blood flow [69] which can lead to increased creatinine clearance [70], and augmented renal drug elimination.

High cardiac indices in combination with fluid therapy and vasopressor support have been demonstrated to enhance renal CL, with resultant increased creatinine clearances and glomerular filtration rates (GFR) [24, 25, 71]. As such, ARC has the potential to produce sub-therapeutic levels of administered medications, particularly when administered in standard doses and/or with standard dosing intervals [66]. Subgroups of critically ill patients, other than those with sepsis, which may also demonstrate ARC include patients with severe trauma [72], traumatic brain injuries [73] and major burn injuries [74].

Acute kidney injury (AKI): The incidence of AKI in the critically ill ranges from 30-70% [75,76], common etiologies include sepsis, drug related toxicity and ischaemia [77-79]. Various classification systems have been created to identify and quantify AKI. The RIFLE classification system is such a system and describes three grades of AKI according to severity; Risk, Injury and Failure. These grades are based on changes in serum creatinine and urine output. The Loss and End Stage Renal Disease (ESRD) are outcome stages and are based on the duration of Renal Replacement Therapy [80].

AKI can influence the pharmacokinetics of drugs through a variety of mechanisms. Typically extravascular fluid accumulation occurs in oligo-anuric AKI [81]. As a result the V_d of hydrophilic drugs can increase considerably. Uremia alters binding of highly protein bound drugs [10] and leads to higher unbound fractions. There is a well documented, although poorly understood, association between AKI and the metabolic enzyme activity in the liver [81-83], resulting in decreased hepatic drug CL.

AKI may lead to a significant decrease in CL of drugs with an expected renal clearance greater than 25-30% of the total body clearance [82]. Decreased GFR, as well as impaired tubular secretion and re-absorption [83, 84] may be responsible this.

Renal replacement therapy (RRT): Up to 65% of patients with AKI, representing ~ 5% of all intensive care admissions, will require RRT during the course of their illness [85,86]. The pharmacokinetics of many drugs are significantly altered by RRT. Drugs usually cleared through the kidney may undergo removal during RRT. The drug properties which will influence clearance by RRT include; the degree of protein binding, the V_d, the molecular weight and the charge of the drug. Various other factors play a role in drug clearance and include; the method of RRT used, intermittent, continuous or a hybrid mode, the mode of clearance, hemofiltration, dialysis or hemodiafiltration and the type of filter used. The dialysis dose, the flow rate and the volume of ultra-filtrate all influence the clearance of drugs excreted across this membrane. A detailed discussion of these changes falls outside the scope of this review, but these factors are well described in the literature [82,87,88].

The hepatobillary system: Liver dysfunction can occur in severe sepsis as part of MODS [89]. Sepsis can cause a decrease in hepatic blood flow which may alter drug metabolism. This is especially important for drugs with high a hepatic extraction ratio, which is dependent on hepatic blood flow and where hepatic perfusion is the rate-limiting process in drug metabolism. Decreased hepatic blood flow may decrease the clearance of these agents [90].

Hypoalbuminaemia, due to decreased syntheses, can cause significant pharmacokinetic effects. Medications that are usually highly protein bound, will have a higher free fraction during hypoalbuminaemic states. Importantly, it is the free fraction of a drug that distributes through-out tissues, thereby resulting in an increased V_d [9]. In a similar fashion, hypoalbuminemia is associated with increased renal clearance [4] for those agents that are highly protein bound, due to greater filtration of free drug across the glomerular membrane [91]. In the case of hepatic elimination, only free drug can diffuse into the hepatocyte, such that the rate of hepatic clearance of agents with a low extraction ratio correlates with the free drug fraction.

During critical illness the liver synthesizes acute phase proteins such as α1-acid glycoprotein (AAG). Basic drugs, such as lignocaine and propanolol, primarily bind to AAG and the free fraction of these drugs can be reduced during stressed states, due to increased binding. Significant inhibition of the cytochrome P-450 (CYP450) isoenzymes [28, 89] is also seen in the critically ill. This causes a decrease in the metabolism of drugs undergoing phase I metabolism. Contributing factors include poor drug diffusion into the hepatocyte, slow drug dissociation from blood components and poor biliary transport [90].

Other systems: gastrointestinal system (GI system) and skin

During shock states blood flow is directed from non-vital organs such as the kidney, spleen, GI system and skin towards vital organs such as the brain, myocardium and lungs. Decreased GI perfusion can cause GI dysfunction with a reduction in its absorptive capacity [92,93] and may result in poor absorption of enterally administered medication. Similar concerns have been raised by Priglinger et al. about the absorption of subcutaneously administered enoxaparin as prophylactic thromboprophylaxis in the critically ill [94].

Methods to Investigate Altered Pharmacokinetics and Organ Function in the Critically Ill

Measuring cardiac output

Given the significant changes encountered in the cardiovascular system in critically ill patients, and the potential impact on drug PK, some assessment of cardiac output is essential in future study design. Various invasive, minimally invasive, and non-invasive techniques exist.

Trans-thoracic Echocardiography (TTE) is a fast and non-invasive technique to assess cardiac output in the ICU setting. CO is calculated using heart rate and stroke volume (derived from the left ventricular outflow tract cross-sectional area and the blood flow velocity through it [95]. Additional information that can be obtained from a bedside TTE include assessment of the left and right ventricular size and function, identification of pericardial effusions and assessment of fluid responsiveness [96]. However, CO monitoring using TTE requires a skilled operator and is unsuitable for continuous cardiac output monitoring.

Pulse Contour Cardiac Output (PiCCO) estimates CO based on a pulse contour analysis, using transpulmonary thermodilution for calibration. It gives continuous cardiac output monitoring based on a beat-to-beat stroke volume estimation. The PiCCO device is connected to a large vessel arterial catheter, as well as a central venous line, which is used for the injection of a thermo-indicator solution. The blood temperature changes are sensed by a thermistor tipped sensor in the arterial catheter. This thermodilution calibration should be done frequently to ensure accuracy and it needs to be performed manually. PiCCO also provides information on additional parameters such as extravascular lung water volume and intrathoracic blood volume (as an estimate of ventricular filling) [97].

Vigileo/Flowtrac (®Edwards Lifesciences) uses the arterial waveform and patient demographics such as patient age, weight, height and gender to calculate continuous CO. It has an internal calibration system, does not require a central venous catheter, nor manual calibrations, and can be connected to a standard intra-arterial catheter. It may become inaccurate during arrhythmias and in patients with significant aortic stenosis [95].

The pulmonary artery catheter (PAC) has long been regarded as the 'Gold Standard' for measuring CO. It uses a thermodilution technique and calculates CO from the modified Steward-Hamilton equation. It can also give information about pulmonary artery pressure, pulmonary artery wedge pressure and oxygenation parameters, such as mixed venous oxygen saturation [98]. It is however invasive, requiring cardiac catheterization by a skilled clinician, and has the potential for serious complications including arrhythmias, valvular lesions and rupture of the pulmonary artery [97]. It may become inaccurate in certain states, such as those associated with a low cardiac output or increased pleural pressures, commonly seen during mechanical ventilation [95].

Measuring body compartment volumes

As previously outlined, changes in the V_d of many agents is likely in the critically ill patient, due to intravenous fluid loading, 'third-spacing', and changes in protein binding. Attempts to measure changes in body fluid compartments, and the subsequent effects on key PK parameters, are highly valuable in informing future study design and empirical dosing schedules. Bio-impedance and tracer dilution techniques are two methods currently being employed in clinical PK studies.

Bioelectrical Impedance is a non invasive estimate of fluid compartment volumes, and is based on knowledge of the electrical properties of tissues. Electrodes are superficially attached to the limbs, with the technique being portable, safe, and reproducible. Changes in tissue conductivity (which is directly proportional to the amount of electrolyte-containing fluid) in response to a small bioelectrical current is then used to provide a rough measure of total body water and extracellular volume [99]. Specifically, at low frequencies, current flows primarily through extracellular fluid which has a low reactance. In contrast, at higher frequencies, the current can penetrate all cells, with an increase in reactance [100]. Although potentially more accurate in healthy subjects, results in critically ill patients remain inconsistent [101,102].

Sodium Bromide (Br) is an anion tracer which distributes into extracellular water, without intra-cellular penetration. By measuring the plasma Bromide concentration after the administration of a known amount, the Corrected Bromide Space (CBS) can be calculated. CBS correlates very closely with extracellular water volume [103].

Indocyanine green (ICG) can be used to measure plasma volume. ICG is a water-soluble tracer that rapidly binds to plasma proteins, especially albumin after intravenous administration. ICG stays in plasma without further distribution and is exclusively eliminated via the liver (by secretion into bile in its unchanged form). As such, following intravenous administration of a known amount, conclusions about both plasma volume [104] and hepatic function can be drawn by monitoring ICG concentrations, and its rate of disappearance from plasma [105]. Although the gold standard application of this technique requires serial blood sampling, ICG concentrations can also be measured continuously, using a non-invasive transcutaneous system attached to the patients finger [104]. This relies on the optical properties of ICG, with near infrared absorption at 805 nm and fluorescence at 890 nm. Good correlation has been demonstrated between each method [106,107].

Measuring tissue penetration

Drugs act to exert their effects at different sites, generally outside the vascular space. In this respect, plasma concentrations alone may not reflect therapeutic concentrations at the site of action. Adequate organ and tissue penetration is often a crucial requirement for efficacy, and assessment of such, should be regularly considered in PK or toxicological study design.

Microdialysis is a semi-invasive sampling technique employed to directly measure unbound drug concentrations in the interstitial space. It involves the implantation of a small probe into the tissue or organ of interest. The probe itself is a short piece of plastic with a semi-permeable lining at its tip, connected to inlet and outlet tubing respectively. The inlet tube is perfused with an aqueous solution at a low and constant flow rate. Solute in the extracellular water is then able to diffuse through the semi-permeable membrane, and its concentration can be subsequently measured by analyzing the fluid collected from the outlet tube [108]. The technique requires *in vivo* calibration, and is limited to primarily small molecular weight hydrophyllic species [109].

Side-stream Dark Field Imaging (SDF) is an imaging modality, recently developed by Ince et al. [32], capable of providing continuous, recordable video of the microcirculation. Green light-emitting diodes (at a wavelength absorbed by hemoglobin), along with a high powered hand-held microscope allows direct visualization of red blood cells within capillaries, venules and arterioles. The most commonly employed site in clinical research is the sub-lingual area. Vessel

density and flow within small and large vessels can be semi-quantified, providing an index of microcirculatory function. Although yet to be tested, poor drug penetration is likely to correlated with a dysfunctional microcirculation.

Measuring clearance

Excretory organ function can be significantly altered in the critically ill, with both increased and impaired function being described. Given the potential effects on half-life, and duration of effect, robust measures of organ function (rather than just injury) should be employed.

A measured Creatinine Clearance (CrCl) provides real-time information about the GFR, and can be calculated from the plasma creatinine concentration and a timed urinary creatinine collection. Creatinine is a small molecular weight waste product of protein metabolism that undergoes glomerular filtration, and tubular secretion, the latter becoming more important with increasing renal dysfunction. Shorter urinary collections [110] may be just as accurate as 8, 12 or 24 hour periods [111], can be repeated regularly to provide information on dynamic changes in renal function, and are likely to have improved compliance. Importantly, a measured CrCl provides more reliable information than isolated plasma creatinine concentrations alone in assessing GFR in the critically ill [112], and has improved accuracy when compared with mathematical estimates (such as the Cockcroft-Gault formula) in the setting of augmented renal clearance [113].

Inulin is filtered through the glomerulus and neither secreted nor reabsorbed in the renal tubule, making it an ideal marker to estimate GFR. Plasma- and timed urinary concentrations are used to determine inulin clearance, although its' poor solubility (and subsequent difficulty in administration) limits its clinical use. Sinistrin, a polyfructose marker, can be used instead of Inulin, as it is easier to administer due to increased solubility [114].

p-Aminohippuric acid (PAH) is both filtered by the glomerulus and undergoes active tubular secretion, with near complete extraction by the kidneys. In this respect it has been traditionally used as a marker of renal plasma flow. Although sampling from the renal vein (to determine an accurate extraction ratio) has often been considered the gold standard methodology, newer techniques utilizing a single intravenous bolus, and limited sampling strategies have been described [115]. Doppler ultrasonography of renal blood flow can be used to calculate the renal resistive index which correlates well with renal vascular resistance and may help to identify those patients in septic shock which will develop AKI [116]. Use in PK studies is currently limited.

Finding a single marker to assess tubular function remains problematic. Neutrophil gelatinase-associated lipocalin (NGAL) is an emerging biomarker of acute kidney injury [117], but provides limited information about tubular function *per se*. Of recent interest is the approach described by Tett et al., where a 'cocktail of makers' have been employed to assess tubular cation secretion, tubular anion secretion and tubular re-absorption concurrently [114]. Such methodology has yet to be assessed in the critically ill.

As previously discussed, indocyanine green is a synthetic highly protein bound dye that can be used to measure hepatic blood flow and liver function [118]. As ICG is rapidly extracted from plasma only by the liver, and enterohepatic re-circulation does not occur, hepatic blood flow, hepatic extraction and biliary transport primarily determine clearance. In this respect, ICG dilution provides a robust estimate of global liver function [119], with prior literature supporting its use in assessing hepatic donor graft function [120].

Bioanalytical methods

Pharmacokinetic clinical trials almost always involve measuring drug levels in plasma - a field known as bioanalysis, which also encompasses assaying metabolites, pro-drugs and endogenous molecules (the analytes) in biological samples such as serum, whole blood, urine, cerebrospinal fluid (CSF), bile, bone, etc as well as non-bodily fluids like dialysis fluid (the matrix). The mainstay of bioanalysis over recent decades has been HPLC - high performance liquid chromatography. The instrument in its simplest form comprises a pump to push the mobile phase at high pressure, an autosampler to inject the sample, a column containing stationary phase to separate the analyte from interference, and a detector to produce a signal proportional to the concentration of analyte.

Assays using a HPLC system with a ultraviolet (UV) detector typically measure microgram-per-millilitre (μg/mL, or mg/L) concentrations of drug, from a plasma sample of 0.1 to 1 millilitre. This arrangement is suitable for pharmacokinetic analysis of many drugs that absorb light at ultraviolet wavelengths, such as paracetamol and certain antibiotics [121]. A smaller number of drugs fluoresce (e.g. ciprofloxacin [122]), and a fluorescence detector on the HPLC will give a stronger signal than UV, such that nanogram per millilitre (ng/mL, or μg/L) can be more readily attained. With a large gain in signal and fewer fluorescent interferences, fluorescence detection is a powerful HPLC technique.

Whilst many drugs do not fluoresce, some, such as gentamicin, do not even possess a suitable UV spectrum. To perform traditional spectrophotometric HPLC analysis with these drugs requires a derivatisation step to chemically react the molecule with a fluorescent tag, usually as a pre-treatment of the sample before being submitted to the HPLC [123]. Whilst this can provide a sensitive method, the process can be more labour intensive, and has the added complication of potentially converting metabolites into the same fluorescent species as the intended analyte.

There are many variations to HPLC with alternate detectors such as electrochemical [124] or light scattering. The largest advance in bioanalysis, however, has been the coupling of a mass spectrometer detector in place of a spectophotometric (light) detector. The mass spectrometer has greatly added to the specificity and the sensitivity of bioanalysis. This technique is broadly known as Liquid Chromatography Mass Spectometry (LCMS) and has some fundamental differences to HPLC. Technological advances have made the Mass Spectrometer more sensitive, such that picogram per millilitre [125] and lower levels a can be readily measured with state of the art machines. Some analytes are not well suited to LCMS, including those that are difficult to ionise within the mass spectrometer and those with a variable mass.

From the view-point of PK study design, the extra power of modern LCMS machines can enable reductions in sample volume and consequently novel approaches to pharmacokinetics. One example is microdialysis, where samples of 1-10 microlitres can be readily measured to provide estimates of drug concentration in tissue at the site of infection. Another example is to measure the drug levels in a dried blood spot of approximately 3 microlitres of whole blood. The advantage here is not in gaining any particular advance in pharmacokinetic data, but logistic. Unlike a plasma sample that requires a centrifuge to collect, and freezing for storage and transport, a dried blood spot specimen can be stable at room temperature.

Assays in plasma tend to release drug that is bound to proteins, and so it is the total level of drug that is measured. The gold standard

in determining free levels is to use the technique of equilibrium dialysis in preparation of samples for submission to HPLC or LCMS. However, good estimates can be obtained using appropriate ultracentrifugation filters typically with a 10 or 30 kD molecular weight cut-off. These disposable devices can isolate a small volume of protein-free plasma without releasing the protein-bound drug. The drug level is then measured in the protein-free plasma. Two important consideration in this process are 1) to conduct the filtration at biological temperature (37°C), and 2) to filter no more than 40% of the plasma volume [126].

A central feature of chromatographic methods - HPLC and LCMS - is the ability to resolve the desired drug from its background and focus on a selected molecule. This is particularly powerful with the most commonly applied form of LCMS where a triple quadrupole mass spectral detector is used - termed LC-MS/MS. However, such specificity necessitates that each LCMS analyte is a discrete entity, which is not the case for all drugs, particularly those of biological origin (such as gentamicin). Fortunately there are alternate technologies for measuring such analytes, such as enzymatic or immunological methods. These methods, widely used in pathology, have advantages of high throughput but can suffer from interference. This 'looseness' of a discrete analyte can be advantageous when quantifying the levels of a family of active ingredient. For example, the colourmetric DL-DMAC method estimates the total amount of proanthrocyanidins in cranberry products, the active ingredients that prevent UTIs [127].

The most appropriate bioanalytical method in PK study design largely on the desired application - what you are trying to achieve in terms of drug, matrix, sample volume, expected concentration, and availability of resources. Whatever the technique, bioanalysis industry expectations are that the methodology will be validated, hence establishing that the precision, accuracy and other characteristics of the method are acceptable. Finally, when the clinical samples are actually measured, calibrators and quality controls are to be assayed alongside and acceptance criteria satisfied in order to obtain reliable results [128].

Conclusion

The pathophysiological changes seen in critically ill patients can be profound, with significant potential for altered pharmacokinetics in this setting. This in turn may result in unexpectedly low or toxic drug levels. It is therefore of paramount importance that clinicians are aware of such changes, in order to ensure physiologically sound prescription. Importantly, these PK changes are complex and can be difficult to predict. Different methods to measure and investigate these pathophysiological changes and altered PK parameters have been presented, with newer technologies providing novel means to assess organ function. Future study design should embrace such technology to ensure accurate investigation in this population.

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