Chemistry & Biology Interface, 2014, 4, 3, 176-191

REVIEW PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Do Blood Sampling Sites Affect Pharmacokinetics?

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Received 31March 2014; Accepted 9 May 2014

Abstract: Preclinical and clinical pharmacokinetic (PK) profiling is the bottleneck of the drug development process. PK involves assessment of plasma/serum/blood drug concentration at different time points post drug administration. Despite the practice of blood sampling from different sites, there is no specific rule for selection of sampling site. Analytical method sensitivity, volume and number of samples, animal/human physiological status and subject compliance play a significant role in sampling site selection. Rare validation of the sampling sites often leads to erroneous estimation of PK parameters; therefore, clear-cut information on reproducibility and validity of blood sampling methods is a prerequisite. This review illustrates various commonly used blood sampling sites from different species with emphasis of its impact on the estimation of PK parameters along with justification of the factors responsible for such variations.

Keywords: Pharmacokinetics, Blood sampling site, Arterio-venous, Blood volume, Venipuncture

1. Introduction

Drug research is an un-paralleled multi-disciplinary process aimed for the development of new therapeutic agents to encounter the current baffling medicinal needs [1]. It begins with an exhaustive study of the cause underlying the disease followed by target identification, characterization and its validation [2]. A hypothesis for disease treatment is developed which helps in use of various approaches for developing new chemical entities (NCEs) [3]. Amongst the large number of NCEs, only a few having promising activities are screened for drug development processes. Drug development process comprises of stringent testing and optimization of hits to evaluate the most effective and least toxic ones, on which *in vitro (Latin "vitro"= Glass)* assays are primarily performed, followed by *in vivo*

(Latin "vivo"= Life) studies [2, 4]. In addition to the knowledge of pharmacokinetic (PK) parameters, pharmacological properties are crucial for the drug development and regulatory purposes [4]. Various *in vitro* and *in silico* methods are available for prognostication of *in vivo* PK, but complete dependency on these methods is not recommended when improper *in vitro-in vivo* correlation (IVIVC) exists. It is strongly advised to conduct *in vivo* PK without completely depending on *in vitro* assays [5].

Pharmacokinetics (*Greek "pharmakon" = drug and "kinetikos" = moving, putting in motion*) is a branch of science that provides the mathematical basis to study the time course of absorption, distribution, metabolism, and excretion of the drug in living organisms [6]. Thus, it aids in decreasing the toxicity and increasing the efficacy of the compound. Up to 1985, seven companies owned by UK conducted a retrospective analysis and revealed that ~40% of the NCEs failed in clinic owing to their poor PK [7, 8]. By 2000, knowledge of PK further reduced the drug attrition rate to ~10% [9]. Therefore, careful assessment of PK parameters during early stages of drug development is essential.

Preclinical PK studies are carried out mostly in rodents to predict the *in vivo* efficacy; followed by studies in larger animals for better exploration of PK [10] which facilitates the selection of fewer potential candidates for further development. As a result, failure of compounds in clinical stages (costing hundreds of millions of dollars) [11] can be minimized to a larger extent. Appropriate results can be obtained by determining the concentration of the compound at the site of action. Practically, these sites are not easily accessible hence, drug concentration is commonly estimated in bio-matrices (blood, urine, saliva, tears, and other body fluids) assuming kinetic homogeneity (proportionality of compound's concentration at the site of action with that of bio-matrix). Amongst these body fluids, blood has been the predominantly used and regulatory acknowledged bio-matrix. Whole blood analysis gives compound's concentration in the circulatory system, including the concentration in erythrocytes and/or leucocytes which is missing in plasma samples. Besides this fact, plasma samples are considered superior to whole blood samples as sample extraction and processing of plasma is quite easier. Additionally, plasma extractives possess less endogenous impurities in comparison to whole blood extractives [12]. Sometimes, whole blood is preferred over plasma when there is substantial temperature dependent red blood cells (RBCs) partitioning and also a choice of sample to increase the sensitivity of the assay where blood-to-plasma partition coefficient is greater than two [13]. Since blood is the bio-matrix of choice, authentic results can be obtained if the blood collection procedure is well assured. Blood may be sampled from several sites of animals and human, but, there is no standardized protocol to choose any particular site. This imparts freedom in choosing a blood sampling site depending on the number of samples, required blood volume, physiological status of animal/human, experience of the researcher, type of experiment and species [13]. Thus, the route of sampling varies from researcher to researcher leading to significant differences in PK parameters of drug that in turn affects its safety and usage. Considering the importance of a unified method for sampling site, this review critically discusses several case studies evidencing the effect of the blood sampling site on the PK along with a brief illustration on various blood sampling sites (Figure 1) and methods (Table 1) used for PK studies in different species.

2. Blood Sampling Sites and Methods

2.1 Sampling from animals

Figure 1. Blood sampling sites in animals and human.

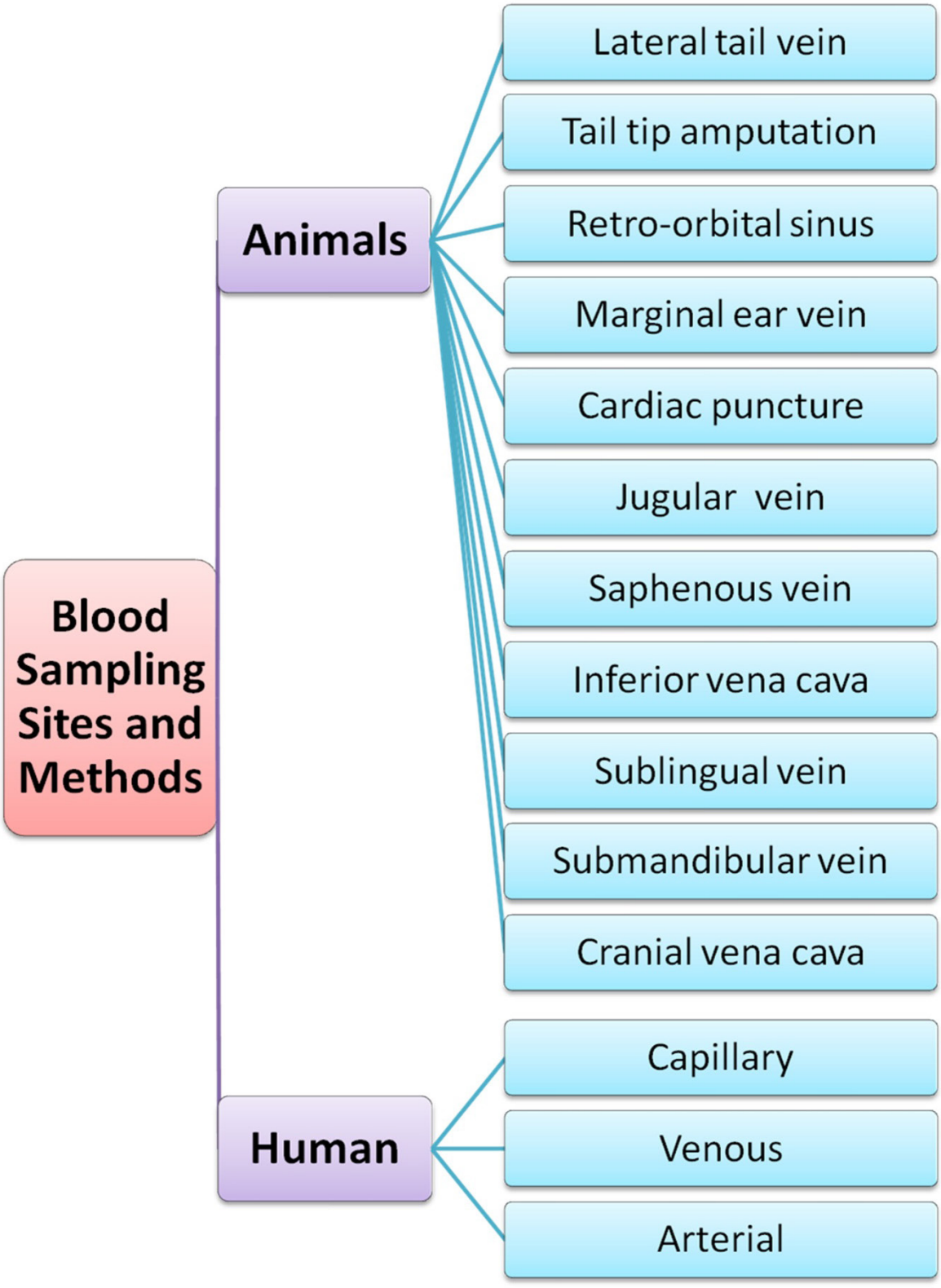
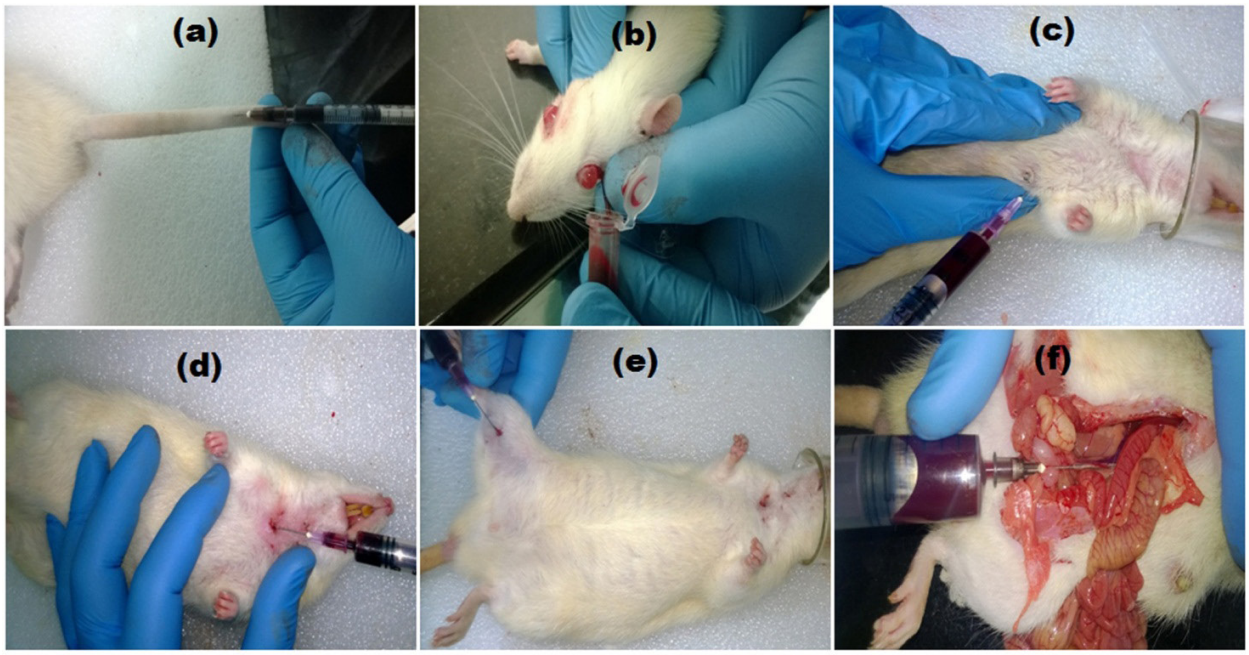


Table 1. Sites of blood sampling and their applicability in animal species [27, 53-56]

Animal	Total Blood Volume (ml/kg)	One time sampling (ml/kg)	Sites of blood sampling										
			Saphenous (lateral tarsal) vein (ml)	Marginal ear vein/ central ear artery (ml)	Sublingual vein (ml)	Lateral tail vein (ml)	Amputation of the tail tip (ml)	Cardiac puncture (ml)	Retro-orbital sinus (ml)	Sub-mandibular vein (ml)	Jugular vein (ml)	Cranial vena cava (ml)	Inferior vena cava (ml)
Rat	64	5.5	0.4	NA	0.2-1	0.2-0.4	0.1-0.2	5-15	0.5-1	0.3	2	0.8-2.5	5-15
Mouse	90	7.7	0.2-0.3	NA	0.3	0.05-1	0.01	0.8-1	0.2-0.3	0.2-0.5	0.2-0.3	NA	0.8
Hamster	87	5.5	NA	NA	0.75	NA	NA	5	0.1-0.5	NA	1	0.2-1.5	5
Guinea pig	75	7.7	0.5	NA	NA	NA	NA	5	NA	NA	2.5	NA	10-15
Rabbit	56	7.7	NA	0.5-10	NA	NA	NA	60-200	NA	NA	NA	NA	NA
Dog	86	9.9	2-5	NA	NA	NA	NA	NA	NA	NA	2-20	NA	NA
Horse	75	8.8	NA	NA	NA	NA	NA	NA	NA	NA	9-12	NA	NA
Monkey	77	6.6	5-10	NA	NA	NA	NA	NA	NA	NA	5-10	NA	NA

NA= not available

Figure 2. Rat blood sampling sites: (a) Lateral tail vein, (b) Retro-orbital sinus, (c) Cardiac puncture, (d) Jugular vein, (e) Saphenous (lateral tarsal) vein, and (f) Inferior vena cava.



2.1.1 Tail vein

2.1.1.1 Lateral tail vein

Sampling of blood from a lateral tail vein of rodents can be accomplished either by restraining the animal in a suitable restrainer or under light anesthesia (for collecting blood in anesthetized condition, before 30 minutes of sampling apply a local anesthetic cream on tail). The blood vessel has to be dilated prior to blood sampling, which can be performed by warming the animal under the low watt light bulb (100 watt) or by keeping the animal cage on a heating pad or in an incubator at a 39 ºC for 5-10 minutes (referred as animal warming methods) [14]. A tourniquet can be applied at the base of the tail. Initially, the tail can be massaged to increase the blood flow in tail vein. In case of hematological analysis, massaging is not recommended as it may cause leucocytosis due to mixing of white blood cells in the sample or it can contaminate the sample with tissue fluid [15]. Following wiping of the tail with an alcohol swab, needle (25-27 G) should be inserted into the lateral tail vein (Figure 2) [16]. The blood is then withdrawn slowly from the vein by negative pressure in the syringe. After blood sampling, a dried gauze should be applied with slight pressure until bleeding stops or cauterizing agent like a styptic pencil (silver nitrate) can be used to attain hemostasis (referred as hemostasis method) [17]. Then, the animal is released from restrainer and observed until it recovers from anesthesia. Cannulation of tail vein can be used for repetitive blood sampling [18].

2.1.1.2 Amputation of the tail tip

The initial procedures (animal warming method and massage) for the amputation of tail tip are same as described in lateral tail vein sampling method. After performing these initial procedures, the tail can be wiped with an alcohol swab and amputation of tail tip up to 1-2 mm (not more than 5 mm over time; cut only fleshy part of tail tip and not any skeletal structures) can be performed through scalpel/straight edge razor or sharp scissors. If blood is not visible on the first attempt, tail tip can have another cut of 2-3 mm [19]. After blood sampling, dried gauze should be applied with slight pressure until the bleeding stops (or apply hemostatic agent) [17]. Then, the animal is released from restrainer. For withdrawal of an additional sample, clotted blood should be removed. Tail should not be excised too long repetitively as this may result in shortening of the tail and trauma to the cartilage/coccygeal vertebrae which are not acceptable [19]. A limitation of this method is that, the blood collected from the tail tip is a mixture of arterial, venous and tissue fluids, so drug levels may differ from systemic circulation levels.

2.1.2 Retro-orbital sinus

Blood collection via retro-orbital sinus is considered as unethical procedure due to its undesirable effects on eyesight of the animal as it causes excessive pressure on the back of the eye that can damage the tissue and produce hematoma which is quite painful for the animal. It may also cause corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia, proptosis of the globe and panophthalmitis. The penetration of micropipette can fracture the fragile bones of the orbit and damage the optic nerve that may lead to blindness [14, 19]. Before sampling, animal must be put under general anesthesia, then instill drops of topical anesthetic agent (e.g., 2% cocaine) [20]. As shown in Figure 2, anesthetized animal is kept aside on the table or hold by hand, such as its head is pointed down. For protruding out the eyeball from the socket, skin is pulled away from the eyeball by placing finger above and thumb below the eye. The edges of the capillary tube should be

checked prior to insertion as rough edges may damage the tissue. Also, capillary tube should be sterilized to avoid any infection. At an angle of 30-45º to the nose, the capillary tube (0.5 mm)/Pasteur pipette is inserted into the corner of the orbital cavity by gently rotating it with downward pressure. The pressure is released when the vein is broken and blood enters into the pipette/tube, after which pipette/tube is withdrawn slightly to get filled. The pipette is removed by covering the open end of the pipette with finger to reduce dropping out the blood [17]. When the required amount of blood is sampled, the tube is removed and the skin of the eyelid is pulled together with applying gentle pressure until bleeding stops. Blood present on skin around eye should be wiped with dry gauze. The animal should be monitored for 30 minutes for any bleeding/swelling [21]. Both the eyes should be used alternatively for repeated sampling and a minimum interval of 21 days is required to recover the affected tissue for collection of the blood from the same site [14].

2.1.3 Marginal ear vein/central ear artery

Blood collection from a marginal ear vein is extremely common in the preclinical PK study in rabbits. Before collecting the blood from ear vein, the animal should be kept in a suitable cage followed by animal warming methods. Prior to blood sampling, fur on the ear should be shaved by electrical or chemical hair remover for clear visibility of the vein. During hair removing, care should be taken as electrical hair shaver may mutilate the skin integrity and chemical hair remover can interact with the administered drug leading to altered PK parameters, and then ethanol and xylene are applied for sterilization and proper vein visualization, respectively. Local anesthetic cream can be applied before 30 minutes of sampling [19]. A needle (21-26 G) should be inserted in parallel to the blood vessel (for marginal ear vein, insert needle at the margin of the ear, for central ear artery insert needle toward the base of the ear up to 10 mm) [22, 23]. If the blood vessel collapses, the ear should be stroked lightly until it gets relaxed and blood flow restarts. Disruption in blood flow is potentially harmful which leads to thrombosis, ischemia and sloughing of pinnal tissue [23]. After blood sampling, dried gauze is applied with slight pressure until the bleeding stops (as mentioned above) and the animal is released from cage [14]. For serial sampling, the needle can be inserted at the same site or other sites on the marginal vein.

2.1.4 Cardiac puncture

Blood sampling by cardiac puncture is used for collection of higher blood volumes and can act as a terminal method of blood collection. Apart from being painful technique, it also causes abnormalities like pericardial bleeding and cardiac tamponade [19]. It may cause serious threat to the animal; therefore, expertise is required for blood collection through cardiac puncture. In the blood sampling, the animal is deeply anesthetized and checked for the spontaneous movement, breathing rate and response stimuli by pinching a toe to ensure anesthesia [24]. To avoid collapsing of the heart, blood should be collected from the ventricular region (either left or right). After that, the index finger should be placed without applying any pressure next to the level of the left lower rib where maximum palpitation can be sensed. The heart should be located approximately between the fourth and sixth rib [25]. As shown in Figure 2, a needle sized 20-25 G [22] with syringe should be inserted between two ribs at 45º angles. To create a vacuum inside the syringe, the plunger is slightly pulled and held in the same position, untill the drop of blood comes into the needle. The plunger is slowly pulled to collect the required amount of blood without moving the needle. The blood is withdrawn slowly to avoid collapsing of heart. After blood

sampling, the animal is euthanized immediately [21].

2.1.5 Jugular vein

The jugular veins bring back deoxygenated blood from the head to the heart via the superior vena cava. Prior to blood sampling, anesthetize the animal and then its (rodent) head is grasped between forefinger and middle finger in such a way that these fingers are bent over the shoulders. The thumb and ring finger may cover the lower chest and the ventral side (abdomen and hip) should be wrapped with other hand. Otherwise restrain the animal by placing loop on the limbs and immobilize the animal. The fur of the neck should be gently shaved. Then the skin can be sterilized with an alcohol swab and wiped with xylene so that the blue colored jugular vein becomes visible, which is running from 2 to 4 mm adjacent to the junction of the sternum and clavicle up to the angle of the jaw [21]. As shown in Figure 2, a needle (20-25 G, 1-3 mm deep) [22] with syringe should be inserted through the skin around the middle point between the sternum and shoulder area [17]. To create a vacuum inside the syringe, the plunger is slightly pulled and held until the drop of blood comes into the needle. The plunger should be slowly pulled to collect the required amount of blood without moving the needle. Blood should be withdrawn slowly to avoid collapsing of blood vessel. If blood flow stops, then the needle can be slightly rotated. After blood sampling, dried gauze is applied with slight pressure to stop the bleeding (or apply hemostatic agent) [17]. Once the desired volume of blood is collected, the animal is made free from restrainer and observed until it recovers from anesthesia. This route (jugular vein) accompanied with cannulation is the most preferred method for serial sampling [26]. For blood sampling through the jugular vein, warming of the animal is not required.

2.1.6 Saphenous (lateral tarsal) vein

Lateral saphenous vein runs dorsally and then sideways over the tarsal joint. As shown in Table 1, saphenous vein can be a site of blood sampling from almost all rodent and non-rodent species [27]. Blood from saphenous vein could be collected under light anesthesia, accompanied by one/two persons. Suitable animal warming methods can be applied to dilate blood vessel [14]. For blood sampling, hind leg of animal is extended with hand followed by the sterilization of the skin with an alcohol swab. Petroleum jelly or silicone grease can be applied or the fur is gently shaved to preclude the spreading of blood [14, 28]. A tourniquet can be placed over the leg with soft pressure on the site of blood collection to dilate the blood vessel and make it visible. As shown in Figure 2, the blood vessel can be punctured by a needle of suitable size (22-30 G) [17, 22, 23] with or without using a disposable syringe. Blood can be collected into a hematocrit tube/collection tube by capillary action or through negative pressure by syringe. Following the required volume of blood collection, dried gauze should be applied at the point of needle insertion with slight pressure until the bleeding stops (or apply hemostatic agent) [17]. Then, the animal is released from restrainer and should be kept under observation until it recuperates from anesthesia [21]. For multiple sampling, clotted blood/scab can be removed from sampling site or another limb can be used. This technique is extensively pertinent to PK study when the sampling volume is small and serial blood sampling is required with the least number of animals per study [19].

2.1.7 Inferior/posterior vena cava

The inferior vena cava is also known as posterior vena cava. It is a large vein formed by the joining of the left and right common iliac vein. It is posterior to the abdominal cavity that carries deoxygenated blood from the

lower half of the body into the right atrium of the heart. Blood sampling from inferior vena cava is the terminal method to collect large volumes of blood. The animal must be deeply anesthetized and placed on a dissecting board on its back. A 2 cm long longitudinal incision should be made on a midline of the abdomen through scalpel blade/surgical scissor [14]. To uncover the vertebral column, the skin folds are pulled away and the peritoneum is then opened; the abdominal viscera are also displaced. The inferior vena cava is situated at the right side of the vertebral column (if it is not clearly visible, then clean visceral fat) [29]. The needle (25 G) with a syringe (5 ml/10 ml) should be carefully inserted [17] in the inferior vena cava (Figure 2) up to 0.5 cm (at just above the junction of the common iliac veins) and the plunger is slightly pulled to create a negative pressure inside the syringe and the required volume of blood is collected. The needle plunger should be moved slowly to save the blood from hemolysis and clotting. After blood sampling, the animal must be euthanized immediately [29].

2.1.8 Sublingual vein

Blood sampling from the sublingual vein is a less reported method for PK studies. For blood collection, anesthetized animal is tightly grasped by the loose skin of the neck which causes partial obstruction of jugular and lingual veins. The animal should be kept in a supine position and another person softly pulls out the tongue with a cotton applicator stick and holds it through thumb and forefinger [30]. The mouth can be rinsed with triple distilled water/saline to remove any contaminant/food/excess saliva (done in an inverted position to avoid aspiration) [31]. By the side of the midline, two sublingual veins are clearly visible (near to the base of the tongue) [32]. Any of these can be punctured by hypodermic needle (23-26 G) [14].

For blood sampling, animal should be turned to ventral position and held horizontally above the collection tube. After blood sampling, the needle is removed, and the tongue is cleaned with a cotton tipped applicator stick to remove remaining blood. For additional sampling, blood can be withdrawn by puncturing both the sublingual veins alternatively [32]. After blood sampling, animal should be observed until it recuperates from anesthesia.

2.1.9 Submandibular vein

The blood sampling from the submandibular vein can be accomplished either under general anesthesia or by manual restrains. If manual restrain is preferred then animal's neck should be held with the thumb and index finger to stabilize submandibular vein, whereas base of tail is grasped between little finger and the palm of the hand [33]. Orbital veins, submandibular veins (located on the jaw line, directly below the lateral canthus) and other veins of the facial region join to form the jugular vein. The site of sampling is slightly behind the mandibular joint and at the beginning of jugular vein [23]. Firstly, the skin should be wiped with an alcohol swab. The blood vessel is punctured at 90º angle by needle (18-23 G)/number 11 scalpel or lancets, and then the oozing blood is collected. After blood sampling, the neck skin is released to stop the blood flow and dried gauze is applied with a little compression for less than a minute or apply hemostatic agent to stop the bleeding [34]. The animal should be observed for any complicacy post blood sampling. By removing the clotted blood/scab, additional sample can be collected from the same site. Blood vessel ruptured through scalpel and needles are not preferred as a scalpel is not able to penetrate far enough and needle may be blocked due to clotting [14]. An animal lancet is preferred in which the depth of the puncture is controllable as it makes the procedure easy and results in consistent blood withdrawal. The position of the puncture is crucial to obtain a sufficient

blood volume [33].

2.1.10 Cranial vena cava

From blood collection through cranial vena cava, anesthetized animal should be placed in dorsal recumbency with the forelegs retracted caudally [19]. Skin must be wiped with an alcohol swab. A needle (23-27 G) with syringe can be inserted at 0.3-0.8 cm adjacent to the manubrium and cranial to the first rib. After skin perforation, the needle can be inserted further up to 0.2-1 cm in the same direction until blood starts to fill the syringe [35]. To create a vacuum inside the syringe, the plunger is slightly pulled and hold in the same position until drops of blood aspirates into the needle. If blood is not aspirated, then needle can be pulled out until it is just underneath the skin and the direction can be slightly changed toward the midline. After that the plunger is pulled slowly to collect the required amount of blood without moving the needle.

2.2 Sampling from human [36, 37]

2.2.1 Capillary sampling

The requirement of small blood volumes is accomplished by capillary sampling. The capillary sampling sites are finger, heel and ear lobe. Among these, finger prick is most commonly preferred for adults. Though, finger prick is considered as capillary sampling it consists of arteriolar, venous and capillary blood [38]. For finger prick, the 3rd or 4th finger of the non-dominant hand should be used to avoid thumb and index finger as they contain the thicker callused skin. Also, 5th finger is avoided as it possesses less soft tissue over the bone. Maximum depth for finger prick is 2.4 mm. Selected finger is disinfected and punctured with one continuous and deliberate stroke. The puncture should be just away from the center of the finger and perpendicular to the fingerprint ridges. Wipe off the first drop of blood and collect the blood with gentle massage. Pressure should not be applied, since it dilutes the sample with tissue fluid. After sampling, dried gauze with gentle pressure is applied to stop bleeding.

In infants (age below 6 months and weight 3-10 kg), the blood sampling is done by heel prick with a maximum puncture depth of 2.4 mm. Lateral plantar or medial surface of the heel is used for the sampling. The leg should be held firmly to prevent the random jerking while sampling. The heel is disinfected and then punctured to collect the sample. After sample collection, dried gauze is applied to stop bleeding. Blood can also be sampled by finger prick in infants (age, above 6 months; weight, above 10 kg) with a maximum puncture depth of 1.2 mm (6 months to 8 years) and 2.4 mm (above 8 years). The arm should be extended and held at the wrist joint in such a way that it is secured for sampling. The selected finger is disinfected and punctured. Once the desired blood volume is collected, dried gauze is applied to stop bleeding. Capillary blood sampling accompanied with dried blood spot technology is the pertinent method for PK, toxicokinetic, therapeutic drug monitoring and disease diagnosis [39].

2.2.2 Venous blood sampling (Venipuncture)

Cephalic, basilic, or median cubital veins are mainly used for venipuncture. Among these, median cubital vein, which lies in the cubital fossa superficial to the bacipital aponeurosis, is most commonly used. For sampling from adults, the arm is hyper-extended and the antecubital fossa is examined to locate the vein. A tourniquet is applied 3-4 inches above the site of sampling and the person is asked to form fist so that blood flow to the region is increased. The intended area is disinfected and needle with a syringe is inserted at an angle of 15-30°. The needle should never be inserted at the point where veins diverge. Once the blood

is visible in the syringe, the required volume of blood is withdrawn. After the blood sampling, the tourniquet is removed. The needle is gently withdrawn and dried cotton gauze is firmly placed over sampling site to stop bleeding.

In term neonates, venipuncture is preferred. It causes less pain than heel prick. An arm of the child is extended and the wrist is rhythmically tightened to increase the blood flow. The area of puncture is warmed to increase the blood flow. A tourniquet is applied 3-4 inches above the sampling site. Pocket pen or trans-illuminator can be used to visualize the veins. After disinfection, a needle is inserted 3-5 mm distal to the vein and the required blood volume is slowly withdrawn using 1-5 ml syringe (larger volume syringes may collapse the blood vessel) and needle (not greater than 25 G) with an extension tube (butterfly). Once the desired blood volume is collected, the tourniquet is removed, the needle is withdrawn slowly and dried cotton gauze is applied with gentle pressure to stop the bleeding.

2.2.3 Arterial blood sampling

Radial, brachial and femoral arteries are mainly used for arterial blood sampling. The disadvantages associated with brachial and femoral sampling like poor collateral circulation, difficulty in localization and damages to the structures in close proximity made radial artery a first choice for arterial sampling. Radial artery runs distally on the anterior part of the forearm. It is located by the index finger with the help of palpitation. Once located, the area is disinfected with an alcohol swab and dried. The plunger of the syringe is pulled up to the required level and then the needle is inserted at an angle of 45° by feeling the palpitation. The needle is advanced into the artery until blood flashback appears and the required volume of blood is collected. After blood sampling, the needle with a syringe is withdrawn and dried cotton gauze is placed over the site with firm pressure to stop the bleeding.

3. Effects of Blood Sampling Sites on Pharmacokinetics

Assessment of compound's concentration in the tissue is a tedious task and less preferred hence, both preclinical and clinical PK studies are performed by sampling body fluids like blood and urine [40, 41]. Since, blood is the most commonly used bio-matrix for PK study of drugs, a plethora of methods are available for sampling from rodents among which arterial sampling is assumed as the best method [40-42]. Many studies have evidenced and suggested that prior to bleeding site selection, a cautious assessment regarding potential effects of bleeding site on PK parameters should be carried out [13].

Among various routes available for blood sampling from rat and mice, retro-orbital sinus and tail vein bleeding are commonly used. Intra-cardiac sampling and sampling through cannulation of the arteries are also used to a lesser extent. Now-a-days, a much focused method is tail vein bleeding owing to the easiness and plausibility for serial sampling from a single animal. At the same time, drawbacks with other routes also promote the practice of tail vein bleeding. However, the validity of the data obtained by tail route is in a quest because of the low blood flow rate [42]. Hence, many studies are conducted to estimate the effect of tail vein sampling on the PK as compared to other routes. Hui *et al.* [13] compared the PK profile generated from samples of tail vein bleeding, femoral artery cannulation and retro-orbital sinus bleeding after intravenous (IV) and oral administration of six marketed drugs (pentoxifylline, gemfibrozil, glipizide, methotrexate, clonidine, and fluoxetine) in rats (Table 2). Following IV administration, it was observed that the elimination half-life ($t_{1/2}$) of all the six drugs obtained by tail vein bleeding

Table 2. Effect of sampling sites on pharmacokinetic parameters in rats [13]

Drug	Route of		C_{max} (µg/mL)	AUC_{0-24} (µg h/mL)	$t_{1/2}$ (h)	F (%)
	Administration	Sampling				
Gemfibrozil	PO	Tail	5.2 ± 0.1	29.0 ± 3.4	23.7 ± 14.5	48 ± 8
		Cannula	3.5 ± 0.7	24.9 ± 3.5*	17.2 ± 14.5	63 ± 17
		Retro-orbital	7.5 ± 0.8	41.9 ± 3.4	55.4 ± 14.5	46 ± 6
	IV	Tail		6.0 ± 0.4	18.5 ± 3.4	
		Cannula		3.9 ± 0.5*	25.6 ± 3.4*	
		Retro-orbital		9.1 ± 1.0	7.0 ± 3.4	
Clonidine HCl	PO	Tail	0.003 ± 0.001	0.024 ± 0.003	25.6 ± 23.0	97 ± 17
		Cannula	0.003 ± 0.001	0.024 ± 0.006	27.1 ± 18.8	>100
		Retro-orbital	0.005 ± 0.001	0.027 ± 0.005	32.7 ± 32.6	>100
	IV	Tail		0.0024 ± 0.0002¥Ψ	3.1 ± 1.2	
		Cannula		0.0011±0.0001	0.4 ± 1.2	
		Retro-orbital		0.0013±0.0002	1.4 ± 1.4	
Glipizide	PO	Tail	29 ± 3	308 ± 8	5.0 ± 0.9	>100
		Cannula	40 ± 12	458 ± 152	4.6 ± 0.9	>100
		Retro-orbital	29 ± 2	299 ± 29	6.8 ± 0.9	85 ± 12
	IV	Tail		22 ± 4	12.2 ± 0.6	
		Cannula		17 ± 2*	4.6 ± 0.6	
		Retro-orbital		35 ± 3	5.2 ± 0.6	
Methotrexate	PO	Tail	0.32 ± 0.06	2.00 ± 0.04	3.8 ± 1.1	10 ± 1
		Cannula	0.37 ± 0.05	1.11 ± 0.22	3.0 ± 1.1	9 ± 2
		Retro-orbital	0.32 ± 0.04	2.05 ± 0.56	5.9 ± 1.1	10 ± 3
	IV	Tail		1.9 ± 0.2¥	1.6 ± 0.4	
		Cannula		1.2 ± 0.1*	1.5 ± 0.4	
		Retro-orbital		1.9 ± 0.1	1.0 ± 0.4	
Fluoxetine	PO	Tail	0.110 ± 0.006¥Ψ	1.33 ± 0.03Ψ	4.2 ± 0.2¥Ψ	57 ± 3¥Ψ
		Cannula	0.263 ± 0.013	1.78 ± 0.02	2.9 ± 0.2	90 ± 1
		Retro-orbital	0.291 ± 0.003	2.23 ± 0.31	3.1 ± 0.2	>100
	IV	Tail		0.234 ± 0.010	3.1 ± 0.3	
		Cannula		0.198 ± 0.003	2.5 ± 0.3	
		Retro-orbital		0.201 ± 0.005	2.9 ± 0.3	

*= significant differences found in values of Cannula bleed compared to Retro-orbital, ¥= significant differences found in values of tail bleed compared to Cannula, Ψ= significant differences found in values of tail bleed compared to retro-orbital

Abbreviations: AUC_{0-24} = area under the serum concentration-time curve upto 24 h, C_{max} = peak concentration, $t_{1/2}$ = elimination half-life, F = bioavailability

method were not significantly different to that of the femoral artery cannula and retro-orbital sinus bleeding methods, but $t_{1/2}$ of gemfibrozil calculated by femoral artery cannula bled sample was significantly different than that of retro-orbital sinus blood samples. Exposures of clonidine were 2-fold higher than that after tail vein bleeding as compared to femoral artery cannula or retro-orbital sinus bleeding. Minor differences in exposures of gemfibrozil, glipizide and methotrexate were observed in femoral artery cannula bled samples as compared to retro-orbital sinus bled samples. Following oral administration, $t_{1/2}$ of all the compounds obtained from the femoral artery cannula bleeding samples was insignificantly different than that of retro-orbital sinus bled samples. Also, fluoxetine $t_{1/2}$ calculated from a tail vein bled samples was higher than that obtained from the femoral artery cannula or retro-orbital bled samples. The statistical difference was observed in the values of C_{max} and $AUC_{0–24}$ of gemfibrozil and fluoxetine. The AUC_{0-24} of gemfibrozil obtained by retro-orbital sinus bled samples was 1.7-fold higher than that calculated from the femoral artery cannula bled samples. The C_{max} and AUC_{0-24} of fluoxetine obtained by femoral artery cannula or retro-orbital sinus bleeding were 1.7 and 2-fold higher than the respective values obtained by tail vein bleeding. These results demonstrated that the PK parameters obtained from the tail vein bleeding of most of the compounds are almost similar to that obtained from femoral-artery cannula or retro-orbital sinus bleeding. Fluoxetine showed significant difference that may be due to its effect on thermoregulatory responses associated with the reduced tail blood flow. Further, Hassana *et al.* [43] compared the PK parameters of cyclophosphamide in mice obtained from samples of retro-orbital sinus and tail vein and revealed that there was no significant difference between the two samples (Table 3).

Johannessen *et al.* [42] observed differences in the PK of antipyrine and acetaminophen in rats following tail amputation and femoral artery sampling (Table 4). They found that the $t_{1/2}$ of antipyrine calculated from tail vein data was 47.5% higher and apparent elimination rate and total clearance were lower by 24% and 29.5%, respectively, as compared to arterial data. The PK parameters of acetaminophen from the tail vein bled samples showed that $t_{1/2}$ was 46% higher and volume of distribution was 36% lower than that from arterial bled samples. These experiments demonstrated that the PK parameters obtained by tail vein samples are significantly different from that obtained by femoral artery and retro-orbital sinus.

Though there are many blood sampling sites available, almost all of the samples are either from arteries or veins. Hence, it is important to evaluate the arterio-venous differences, if present. Darwish *et al.* [44] observed higher fentanyl concentration in arterial samples as compared to venous samples (Table 5). This difference was expected pertaining to significant early distribution of fentanyl from plasma to highly perfused tissues. Bojholm *et al*. [45] investigated arterio-venous differences of four radiolabelled antiepileptic drugs (phenobarbital, phenytoin, clonazepam and diazepam) following a rapid IV bolus administration in neurologically disordered patients. A similar study was carried out by Shin *et al.* [46] after a rapid IV bolus administration of vancomycin to rabbits. The initial arterial concentrations of all the drugs were much higher than respective venous concentrations. The studies revealed that arterial drug concentration best described the blood concentration of drug in the initial phase after IV administration and was suitable in determining initial drug distribution.

Considering the various sampling sites from humans, capillary sampling is the most preferred route of blood sampling for the estimation

Table 3. Effect of sampling sites on disposition of cyclophosphamide on intra-peritoneal administration in mice [43]

Route of sampling	C_{max} (µg/mL)	AUC_{0-t} (µg h/mL)	V_{ss} (mL)	Clearance (mL/h)
Tail (serial sampling)	108.8 ± 18.2	68.9 ± 10.5	44 ± 9	63 ± 6
Retro-orbital	81.3 ± 11.2	61.3 ± 9.8	60 ± 6	80 ± 9
Tail	78.7 ± 24.6	56.6 ± 13.8	65 ± 15	88 ± 18

Abbreviations: AUC_{0-t} = area under the serum concentration-time curve upto last sampling time, C_{max} = peak concentration, V_{ss} = volume of distribution at steady-state

Table 4. Effect of sampling sites on disposition of drugs after intravenous administration in rats [42]

Drug	Route of sampling	Clearance (ml min^{-1} kg^{-1})	Terminal elimination rate (β; 10^{-3} min^{-1})	$t_{1/2,\beta}$ (min)	V_d (L Kg^{-1})
Antipyrine	Tail	4.5 ± 0.6@	4.1 ± 0.6@	194 ± 37@	1.15 ± 0.13
	Artery	6.4 ± 0.4	5.4 ± 0.4	131 ± 11	1.18 ± 0.06
Acetaminophen	Tail	13.1 ± 2.0	21.2 ± 1.4@	33.4 ± 2.6@	0.60 ± 0.09@
	Artery	12.5 ± 1.0	33.0 ± 2.5	21.6 ± 1.8	0.39 ± 0.04

@Significantly different from arterial data ($p < 0.05$)
Abbreviations: $t_{1/2,\beta}$ = terminal elimination half-life, V_d = volume of distribution

Table 5. Effect of sampling sites on systemic availability of fentanyl on buccal administration in human [44]

Route of sampling	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng h/mL)	AUC_{tmax} (ng h/mL)
Arterial	1.62 ± 0.56	0.48 (0.25-1.48)	3.00 ± 0.93	0.72 ± 0.23
Venous	1.03 ± 0.37	0.73 (0.48-1.98)	2.56 ± 0.85	0.32 ± 0.15

Abbreviations: AUC_{0-t} = area under the serum concentration-time curve upto last sampling time, AUC_{tmax} = AUC from time zero to median t_{max}, C_{max} = peak concentration, t_{max} = time to C_{max}

Table 6. Effect of sampling sites on disposition of Compound A in dog [49]

Route of administration	Route of sampling	C_{max} (µmol/L)	AUC_{0-t} (µmol h/L)	Clearance (L/h/kg)	F (%)
PO		0.014	<0.040	-	<2
IV	Jugular vein	1.7	2.1	3.1	-
Sublingual		0.90	0.89	-	42
	Saphenous vein	0.21	0.41	-	19

Abbreviations: AUC_{0-t} = area under the serum concentration-time curve upto last sampling time, C_{max} = peak concentration, F = Bioavailability

Table 7. Effect of sampling sites on disposition of buprenorphine in horse [40]

Route of administration	Route of sampling	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng h/mL)	MRT(h)	Clearance (mL/kg/min)
IV	Jugular	-	16.55 ± 2.26	7.89 ± 2.50	6.13 ± 0.86
		8.77 ± 3.19	22.49 ± 10.72	-	-
Sublingual	Thoracic	-	-	-	-
	Jugular	10.28 ± 4.59	-	-	-

Abbreviations: AUC_{0-t} = area under the serum concentration-time curve from time zero to infinity, C_{max} = peak concentration, MRT = mean residence time

of drug levels. Minimum invasiveness, easy sample collection, high patient compliance, most preferred in the case of infants, and very importantly enabling serial sampling, where regular monitoring of drugs is needed are some of the advantages with capillary sampling. The aforesaid advantages lead to the replacement of venous sampling by finger prick. Webb *et al.* [47] and Pettersen *et al.* [48] demonstrated a significant correlation between finger prick and venous samples which supported the fact of replacement. Contrarily, Mohammed *et al.* [38] reported a notable difference in paracetamol levels obtained by finger prick and venous samples which were prominent in the absorption phase before the attainment of distribution equilibrium.

For the past few decades, there is an ever-increasing concern about trans-mucosal drug delivery (namely sublingual, buccal, and nasal) as it is one of the means to achieve high bioavailability by bypassing the first-pass metabolism [49]. Unlike human, the presence of the completely keratinized oral lining in several animals limits their usage for preclinical studies of transmucosal drug delivery. Among rodents, rabbits are the only animals with non-keratinized mucosal tissue similar to human. However, larger animals such as monkeys, pigs, dogs are also ideal [50]. Following transmucosal drug delivery, there is a risk of over-estimation of drug concentration in blood when collected from the jugular vein. This is due to linguo-facial vein mediated drainage from oral cavity to the jugular vein. Many studies have been performed to evaluate the impact of jugular vein sampling following transmucosal dose in head region. Sohlberg *et al.* [49] studied the impact of site of blood sampling on PK parameters following administration of sublingual dose of compound A (synthesized by AstraZeneca) to dogs. When the results of jugular vein were compared with that of leg vein, bioavailability and C_{max} of the compound was found to be increased by 2 and 4-fold, respectively (Table 6). Messenger *et al.* [40] observed significant differences in PK parameters estimated from the jugular vein than that of thoracic vein sampling after sublingual and IV administration of buprenorphine to horses (Table 7) and suggested that jugular vein sampling should not be considered as a route for assessing PK parameters following a sublingual dose. The effect of the blood sampling site on the PK of insulin, nicotine, and morphine after IV and nasal administration to sheep was studied by Illum *et al.* [51]. They found that sampling from the jugular vein and carotid artery resulted in similar C_{max}, t_{max} and AUC values without any significant differences after IV and nasal administration of insulin and IV administration of morphine. On the contrary, significant differences were noticed after nasal administration of morphine. The C_{max}, t_{max} and AUC values showed significant variations on jugular and cephalic vein sampling, which were

minute in case of IV administration and more pronounced with nasal delivery. After nasal administration, jugular vein sampling resulted in an overestimation of parameters (C_{max}, t_{max}, AUC and bioavailability) of morphine and nicotine in comparison with carotid artery and cephalic vein, respectively. Nicotine and morphine possess a high V_d which may be a plausible reason for the overestimation of results. Whereas, insulin possesses a low V_d hence, it remained unaffected with alteration in sampling site.

The impact of arterial and venous sampling on pharmacodynamic parameters of morphine-6-glucuronide (M6G) was evaluated by Olofsen *et al.* [52] following IV administration in healthy human volunteers. They concluded that owing to the large differences in arterial-venous concentrations of M6G, the pharmacodynamic estimates get biased. As a result, potency and $t_{1/2}$ were likely to be overestimated by 40% and 30%, respectively.

Based on the above mentioned studies, several outcomes can be drawn such as: (i) drugs that effect thermoregulation cause alteration in the rate of blood flow to the tail, hence, tail vein bleeding for such drugs should be avoided for PK estimation, (ii) jugular vein sampling should be avoided following trans-mucosal delivery of drugs/NCEs since it leads to an overestimation of results. This overestimation may be due to drainage of oral cavity into jugular vein mediated by linguo-facial vein and it is hypothesized that a high V_d of the compound may also result in this overestimation, (iii) arterio-venous differences in the drug levels can be anticipated for drugs having low molecular size, high lipid solubility and low protein binding thus, possessing rapid distribution, (iv) during population PK studies, finger prick method of blood sampling is preferred for serial sampling from patients, especially in case of geriatric and pediatric population. However, this method is always associated with some uncertainty in prediction of accurate PK parameters. For some drugs, there is a plausibility of existence of significant difference between their levels in finger prick and venous blood. These differences are observed as a consequence of arterio-venous differences. All the aforesaid studies have demonstrated that if parameters estimated from one route are considered appropriate then the respective parameters obtained from other sites are in quest for their validity. These studies also stress the importance of sampling site validation prior to site selection.

4. Conclusion

Conventionally, blood is assumed as a homogenous compartment. It is believed that sampling from different sites should not affect the estimated PK parameters. Beholding the above studies, this belief seems to be futile. However, studies have emphasized that consideration must be given to the effect of blood sampling site for estimation of PK parameters. Though different sites of blood sampling are used during preclinical and clinical studies, specific standardized guidelines are not yet established. Special attention of regulatory authorities and researchers is required to develop a guideline for blood sampling site selection during preclinical and clinical PK studies.

Acknowledgments

Author AS is thankful to ICMR, New Delhi, for financial support in the form of his fellowship for the CDRI Communication **8696**.

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