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Review

Use of microdialysis for the assessment of fluoroquinolone pharmacokinetics in the clinical practice

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ABSTRACT

Antibacterial drugs, including fluoroquinolones, can exert their therapeutic action only with adequate penetration at the infection site. Multiple factors, such as rate of protein binding, drug liposolubility and organ blood-flow all influence ability of antibiotics to penetrate target tissues. Microdialysis is an *in vivo* sampling technique that has been successfully applied to measure the distribution of fluoroquinolones in the interstitial fluid of different tissues both in animal studies and clinical setting. Tissue concentrations need to be interpreted within the context of the pathogenesis and causative agents implicated in infections. Integration of microdialysis -derived tissue pharmacokinetics with pharmacodynamic data offers crucial information for correlating exposure with antibacterial effect. This review explores these concepts and provides an overview of tissue concentrations of fluoroquinolones derived from microdialysis studies and explores the therapeutic implications of fluoroquinolone distribution at various target tissues.

1. Introduction

Fluoroquinolones are an important class of broad-spectrum antimicrobials commonly used in both outpatient and hospital setting (Shams and Evans, 2005). They display good *in vitro* activity against the pathogens frequently implicated in respiratory, urinary and skin and soft tissue infections, but antibacterial *in vivo* efficacy cannot be predicted solely on this information (Drusano et al., 2004; Zelenitsky et al., 2003a). Traditional interpretation of *in vitro* antibiotic potency was based on correlating this data with plasma antibiotic levels (Wispelwey, 2005). However, majority of infections are located in peripheral tissues, and tissue concentrations may not necessary correspond to plasma concentration in certain patients and tissue compartments (Lagler and Zeitlinger, 2014). Furthermore, emerging antimicrobial resistance increases the chance of clinical failure despite *in vitro* susceptibility (Labreche and Frei, 2012). Improved characterization of fluoroquinolone tissue pharmacokinetics (PK) (Hurtado et al., 2014; Liu et al., 2014; Marchand et al., 2008; Sammeta et al., 2009) and penetration ratios allows for treatment optimization, and precise information about fluoroquinolone tissue PK is of great importance as drug distribution is frequently nonhomogeneous and tissue specific (Taccone et al., 2016). Tissue pharmacokinetics of antibiotics have been studied in clinical setting using different approaches - indirect modeling of tissue drug levels from plasma drug concentration curves, tissue biopsies, skin blister sampling, microdialysis (Liu and Derendorf, 2003; Mariappan et al., 2013). Various imaging techniques- planar gamma scintigraphy, single photon emission computed tomography, positron emission tomography and magnetic resonance spectroscopy can also be employed to pharmacokinetic studies (Fischman et al., 2002; Langer et al., 2005; Schwameis and Zeitlinger, 2013). These novel images techniques are costly and since radiolabeling of antibiotics was shown to be difficult, a limited number of studies of antibiotic distribution has been conducted using imaging techniques (Schwameis and Zeitlinger, 2013). Common method of taking samples of tissue biopsies and successive quantification in tissue homogenates, produces measures of average concentration over several compartments as tissue homogenate includes drug that is protein bound and unbound, intracellular and extracellular (Edginton et al., 2009; Lorentzen et al., 2000). Majority of

Abbreviations: AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; CABG, coronary artery bypass grafting; CFU, colony forming units; C_{max}, peak concentration; CPB, cardiopulmonary bypass; CSF, cerebrospinal fluid; ELF, epithelial lining fluid; $_f$AUC, free area under the concentration-time curves; ISF, interstitial fluid; MDR-TB, multi-drug resistant tuberculosis; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*; OATPs, organic anion transporting polypeptides; OATs, organic anion transporters -transporting polypeptides; OCTs, organic cation transporters; OPCAB, off-pump coronary artery bypass grafting; p.o., *per os*; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetics; SSI, skin and soft tissue infections; SSI, surgical site infection; tAUC, total area under the concentration-time curve; t_{max}, time to peak concentration

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infections occur in the interstitial fluid, and for fluoroquinolones, this leads to overestimation of concentrations due to pronounced intracellular accumulation (Brunner et al., 1999). Therefore, plasma and whole tissue concentrations do not offer sufficient information for interpretation of fluoroquinolone pharmacokinetics.

Microdialysis is a semi-invasive technique which allows direct sampling of interstitial fluid (ISF) from various tissues (de la Pena et al., 2000). While newer cost-intensive imaging techniques may not be easily applicable in routine clinical settings for PK studies, microdialysis is a technique feasible for studying drug distribution in several human tissues. First developed in the 1960s to measure concentrations of neurotransmitters in rat brain, and gradually adopted in other research for sampling in animal studies (Chaurasia et al., 2007), during last decades with development and refinement of the technique microdialysis has found broad range of applications in clinical setting. With approved microdialysis-catheters available for use in humans, microdialysis was performed in soft tissues, heart, brain, bone and lung for the study of both endogenous compounds and drugs (Hillered et al., 2014). Microdialysis has established particularly useful application in the field of antibacterial agents, as the sampling and monitoring of the antibacterial concentration in the ISF, where most of the infections are located, is easily manageable by microdialysis (Chaurasia et al., 2007; Lagler and Zeitlinger, 2014; Roberts et al., 2015). The outcome of antibiotic treatment depends on several complex interactions between the infectious agent, antimicrobial drug and host defense mechanisms (Jacobs, 2001; Wispelwey, 2005), and treatment outcome shows high variability. Concerning this, microdialysis has brought new opportunities to antibacterials research that will allow better understanding of exposure-response relationships. As microdialysis allows for direct measurement of free, unbound concentration of antibiotics (de la Pena et al., 2000), which are responsible for antibacterial effect, integration of microdialysis-derived tissue pharmacokinetics to pharmacodynamic data offers crucial information for correlating exposure with effect. Drugs within a single drug class and with apparently closely related structures may express marked differences in tissue distribution (Mariappan et al., 2013). This article outlines the principles of microdialysis and its application in estimation of target site concentrations of fluoroquinolones in different tissues. It also explores the therapeutic implications of the results of microdialysis studies.

2. Principles and applications of microdialysis

Microdialysis enables continuous sampling of analytes from the extracellular space. This is achieved through implantation of double lumen catheter with a semi-permeable dialysis membrane which mimics the passive function of a capillary blood vessel (D'Souza et al., 2014). A microdialysis catheter is inserted in target tissue and continuously perfused with physiologically compatible perfusion fluid (perfusate), and depending on a molecular weight cut-off of the membrane of catheter used, only certain analytes diffuse from the ISF into the dialysate across the membrane and may be sampled (Brunner et al., 2005; Chaurasia et al., 2007).

Using membranes with a lower molecular weight cut-off of 20–30 kDa allows the measuring of smaller molecules (*i.e.* most drugs, including antibiotics) but does not allow passing of proteins, cells or cellular debris. Therefore, centrifugation or protein precipitation is not necessary prior to sample analysis. Probes differ in diameter, length and form depending on the tissue compartment studied. Concentric stainless steel cannulas are usually used in neurochemical studies, for sampling soft tissues such as liver, muscle, heart and skin a linear probe can be employed and for blood sampling a flexible probe has been developed (Nandi and Lunte, 2009).

Exchange ensues in both directions across the semi-permeable membrane of the probe depending on the orientation of the solute concentration gradients, and the complete equilibrium is difficult to achieve. Consequently, not all of the analyte present in the ISF will diffuse over the dialysis membrane and be recovered in the perfusate. Rate of recovery depends on membrane composition and length, perfusate type, length of the membrane, flow rate, temperature, and the physical attributes of the analyte. Generally, small water-soluble molecules have high recovery in laboratory testing, and the lower the flow rate, the higher the recovery (Shannon et al., 2013). The surface area of a microdialysis probe can be increased by using probes with bigger length, and not the diameter. Flow rate directly determines the volume of the sample collected (Nandi and Lunte, 2009). Slower flow rates will increase the relative recovery of the analyte, but the collection period will need to be longer to yield a sufficient volume for analytical detection leading to a loss of temporal resolution, since sample volumes of < 1 µl are difficult to manipulate. Higher flow rate and therefore low recoveries, on the other hand, result in less concentrated samples for analysis. Taking all this into account, the analytical methods used for analysis must be carefully developed. Microdialysis typically results in small sample volumes of 1–10 µl, often with low analyte concentration (1 pM–1 µM), (Hansen et al., 1999) which requires analytical techniques with high sensitivity. Several analytical techniques have been successfully employed for quantification of endogenous and exogenous substances in dialysates, including biosensors (Nandi and Lunte, 2009), immunoassays, mass spectrometry and separation-based methods *i.e.* liquid chromatography with UV or fluorescent detection (Veringa et al., 2016). Analytical challenges have been overcome with the introduction and refinement of HPLC coupled with tandem mass spectrometry (LC-MS/MS) (Muller and Rentsch, 2010). LC-MS/MS is a fast, accurate and highly versatile technique employing the individual capabilities of liquid chromatography which separates mixtures with multiple components, and high specificity and detection of mass spectrometry (Veringa et al., 2016; Zestos and Kennedy, 2017).

Another concern is constituted by potential adsorption of analyzed substance to inner surfaces of sampling vials and microdialysis equipment. Study by Matzneller et al. of colistin distribution using microdialysis indicated binding of colistin as a possible reason for its low recovery (Matzneller et al., 2015). Therefore, strenuous *in vitro* experimentations and calibrations are necessary prior to any *in vivo* experiments.

For most analytes the equilibrium between interstitium and the perfusion medium is incomplete, which is why microdialysis probes need to be calibrated. Commonly used method is the retrodyalisis, where microdialysis probes are continuously perfused with solution containing known and constant concentration of studied substance until equilibrium ensues. Afterwards, concentration of the studied substance is determined in the dialysate and relative recovery is calculated as:

$$\textit{Relative recovery}\ [\%] = 100 - 100\,x\frac{\mathrm{C(dyalisate)}}{C\,(\textit{perfusate})}$$

Method of zero net flux is more accurate than retrodyalisis (Hansen et al., 1999), but time consuming. The probes are perfused with fluctuant concentration of studied substance over a period of time, sometimes up to 12 h, until the equilibrium – the match of the perfusate concentration and the ones *in vivo* ensues. Data is plotted and actual sample concentration is determined. At this concentration, there is no net diffusion of the analyte into or out of the microdialysis probe, because the concentration in the sample and in the probe lumen is the same (Nandi and Lunte, 2009).

Microdialysis was applied in studying drug distribution in myriad of tissues in preclinical and clinical settings, including skeletal and heart muscle, skin, blood, bone, adipose tissue, lung, liver, middle ear, spinal cord, eye, synovial fluid, gut lumen, intrathecal and ventricular cerebrospinal fluid, peritoneum and tumorous growth (Chang et al., 2000; DeGuchi et al., 1992; Eisenberg et al., 1993; Hurtado et al., 2014; Kovar et al., 1997; Lu et al., 2007; Luer et al., 2004; Mindermann et al., 1993; Mindermann et al., 1998; Tsai et al., 2000a; Tsai et al., 2000b; Tsai et al., 1999; Waga and Ehinger, 1997). Microdialysis utilization in pre-

Table 1
Details on clinical studies on fluoroquinolone pharmacokinetics employing microdialysis.

Reference, year, special considerations	Antibacterial agent, dose	Tissue examined	Tissue penetration (tissue/plasma ratio)	Causative bacteria, MIC tested	PK/PD values obtained	Main PK/PD index	Main conclusion
Zeitlinger et al. (2003a), patients with sepsis	Levofloxacin 500 mg i.v. infusion over 30 min	Muscle	fAUC ratio = 0.85 ± 0.39 tAUC ratio = 0.55 ± 0.26	*Pseudomonas aeruginosa* (2μg/ml) *Staphylococcus aureus* (0.5μg/ml)	*In vitro* PK/PD simulation with clinical isolates	Significant interindividual differences in bacterial killing rates for *P. Aeuriginosa* based on tissue PK.	Concentrations of the drug in tissue may be inadequate to eradicate less susceptible pathogen. Individual differences in the tissue penetration of levofloxacin may markedly affect target site killing of bacteria for which MICs are close to 2 g/ml.
Bellmann et al. (2004), patients with soft tissue infection	Levofloxacin 500 mg i.v. over 30 min	Subcutaneous adipose tissue – Inflamed (Diabetic patients-margins of the ulcer, nondiabeic patients-hyperaemic zones), Healthy - Subcutaneous adipose tissue in the contralateral thigh.	$fAUC_{(0-10h)}$ Inflamed tissue = 1.2 ± 1.0 Healthy tissue = 1.1 ± 0.6	MIC = 0.5μg/ml	Calculation based on breakpoint value	$AUC_{(0,24\ h)}$/MIC > 100 h	Equilibration between free concentrations of levofloxacin in plasma and those in subcutaneous adipose tissue was unaffected by local inflammation. Adequate levofloxacin target site concentrations were reached. High interindividual variability was noted.
Hutschala et al. (2005), patients undergoing coronary artery bypass grafting	Levofloxacin 500 mg i.v. over 30 min	Lung	tAUC ratio median 0.6 (range 0.4–0.9)	*Pseudomonas aeruginosa*, MIC = 8.0μg/ml	Calculation based on literature data	AUC/MIC = 2.4 (range 1.3–4.2)	Levofloxacin administered with the usual dose is not associated with sufficient lung tissue concentrations to eradicate less susceptible pathogens. No high variation in drug penetration was observed.
Hutschala et al. (2008) and Hutschala et al. (2005), 2008 patients with atelectasis	Levofloxacin 500 mg i.v. over 30 min	Lung – atelectasis Lung - healthy	Lung – atelectasis fAUC ratio = 0.3 (0.1–0.7) Lung - healthy fAUC ratio = 0.7 (0.4–0.8)	*Klebsiella* *Pseudomonas*	Calculation based on literature data	AUC_{tissue}/MIC_{90} Atelectasis 8.7 (6.4–9.8) Healthy tissue 18.2 (15.3–20.1) Atelectasis 2.2 (1.8–2.7) Healthy tissue 5.6 (4.9–6.3)	Atelectasis formations lead to critically lower lung tissue concentrations of levofloxacin in non-dependent parts of lung tissue.
Kempker et al. (2015), cavitary lesions, patients with MDR-TB	750–1000 mg levofloxacin p.o.	Surgicaly removed cavitary lesion (*ex vivo*)	fC ratio = 1.33 (0.63–2.36) Cmax < 8	*Mycobacterium tuberculosis*			Low serum Cmax and AUC values found for the majority of patients. Patient selection may have influenced the results. Drug had good penetration into cavitary tissues.
Zeitlinger et al. (2007), patients undergoing elective lung surgery and healthy volunteers	500 mg i.v. levofloxacin	Lung, skeletal muscle, subcutaneous adipose tissue	AUClung = 4535 (3960–4711) AUCplasma patients = 6750 (5019–10,772) AUCplasmaheathy = 8073 (5001–10,093) AUCmuscle = 8762 (3440–16,389) $AUC_{adiposetissue}$ = 6983 (2645–12,840)	MRSA, MIC = 32 mg/l *Haemophilus influenzae* or *Moraxella catarrhalis* (both MIC = 0.03 mg/l) *Streptococcus pneumoniae* (MIC = 1 mg/l) *S. aureus* (MSSA, MIC = 0.5 mg/l) *Pseudomonas aeruginosa* (MIC = 8 mg/l)	Calculation based on literature data	$fAUC_{0-24}$/MIC 2.36 (2.06–2.45) 2519 (2200–2617) 75.6 (66.0–78.5) 151 (132–157) 9.45 (8.25–9.81),	2-Fold and 1.5-fold higher AUCs0–∞ for the ISF of muscle and adipose tissue compared with lung, respectively. Inadequate PK/PD for MRSA, *P. aeruginosa*.

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Table 1 (*continued*)

Reference, year, special considerations	Antibacterial agent, dose	Tissue examined	Tissue penetration (tissue/plasma ratio)	Causative bacteria, MIC tested	PK/PD values obtained	Main PK/PD index	Main conclusion
Brunner et al. (1999), 1999 healthy volunteers	200 mg i.v. ciprofloxacin	Vastus medialis, subcutaneous adipose tissue	fAUC ratio: Muscle 1.23 ± 0.24 SAD 0.89 ± 0.16	*Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.	*In vitro* PK/PD simulation with clinical isolates	Failure to eradicate the strains *in vitro* occurred with modeling ISF concentrations.	Interstitial ciprofloxacin concentrations were significantly lower than corresponding total serum drug concentrations: applying serum pharmacokinetics overestimated not only the antibiotic concentration but also the antibiotic activity at the target site. Ciprofloxacin concentrations at the site of effect may be sub-inhibitory although effective concentrations are attained in serum
Schuck et al. (2005), 2005 healthy volunteers	250-mg ciprofloxacin p.o.	Thigh muscle	fAUC ratio = 0.92 ± 0.63	Not reported – study examined influence of simulated microgravity on ciprofloxacin tissue penetration.			
Joukhadar et al. (2005), healthy male volunteers, influence of blood flow on tissue penetration	200 mg ciprofloxacin i.v. over 20 min	Subcutaneous adipose tissue of warmed and contralateral nonwarmed lower extremities	Plasma total AUC_{0-5} = 2.81 ± 0.44 Warmed tissue AUC_{0-5} = 3.06 ± 1.37 Reference tissue AUC_{0-5} = 2.17 ± 0.66	*Pseudomonas aeruginosa* (0.5 mg/l and 2.0 mg/l)	*In vitro* PK/PD simulation with clinical isolates and two standard strains	Growth inhibition was 2.0 log10 CFU/ml more effective for the warmed extremity in comparison to that for the reference thigh.	Concentrations of ciprofloxacin in subcutaneous adipose tissue increase significantly by means of local warming of the dermis.
Bielecka-Grzela and Klimowicz (2005), healthy volunteers	500 mg ciprofloxacin p.o. single dose	Skin	tAUC = 0.550 ± 0.150				Maximum concentration of in the cutaneous microdialysate was achieved somewhat later than in plasma. Ciprofloxacin penetrated into skin and achieved concentrations that exceeded the minimum inhibitory concentrations for susceptible pathogens.
Muller et al. (1999a), inflamed tissue of diabetic patients	200 mg ciprofloxacin i.v.	Diabetic ulcer Healthy subcutis	AUCreference/AUCserum ratio was 0.82 ± 6 AUClesion/AUCserum 0.99 ± 0.15 AUClesion/AUCreference ratio was 0.99 ± 6				Inflammation appears to have little or no effect on the penetration of ciprofloxacin into tissue.
Hollenstein et al. (2001), 12 obese and paired control lean subjects	2.85 mg/kg ciprofloxacin i.v.	SAT	Obese tAUC ratio = 0.46 ± 0.27 Lean tAUC ratio = 0.85 ± 0.36				We conclude that the penetration process into the interstitial space fluid is impaired in obese subjects. Weight-adjusted dosing based on actual body weight will yield adequate tissue levels for ciprofloxacin.
Brunner et al. (2002), healthy volunteers	400 mg ciprofloxacin i.v. over 60 min SD 500 mg ciprofloxacin p.o. SD	Muscle SAT	p.o. Muscle 0.57 ± 0.04 Subcutis 0.57 ± 0.09 i.v. Muscle 0.68 ± 0.04 Subcutis 0.57 ± 0.09 for	*Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*	*In vitro* PK/PD simulation with clinical isolates	After a 12-h simulation, the numbers of CFU were below the detection limits for all three strains	The results of the present study indicate that administration of a single dose of two bioequivalent dosage forms of ciprofloxacin might lead to differences in pharmacokinetics at the target site. These differences, however, are not related to differences in pharmacodynamics at the target site

(*continued on next page*)

Table 1 (*continued*)

Reference, year, special considerations	Antibacterial agent, dose	Tissue examined	Tissue penetration (tissue/plasma ratio)	Causative bacteria, MIC tested	PK/PD values obtained	Main PK/PD index	Main conclusion
Joukhadar et al. (2003b), 2003 patients with soft tissue infections	Single i.v. 400 mg moxifloxacin	Subcutaneous adipose tissue	fAUC ratio: Diabetics Healthy 0.9 ± 0.6 Inflamed 0.5 ± 0.4 Nondiabetics Healthy 0.5 ± 0.3 Inflamed 1.2 ± 0.8	*Streptococcus* species (0.25 mg/l) MSSA (0.12 mg/l)	Calculation based on literature data	AUC0–24 $_{fplasma}$/MIC 58–121 h	Time-concentration profile of free moxifloxacin in plasma and tissue were identical. Diabetic patient had lower moxifloxacin concentrations in inflamed tissue. Thus, the pharmacokinetics of moxifloxacin in tissue and plasma support its use for the treatment of STIs in diabetic and nondiabetic patients
Burkhardt et al. (2006), 2006 subcutaneous tissue in patients with spinal cord injury and decubitus ulcers	400 mg/d moxifloxacin p.o.	Subcutaneous adipose tissue	fAUC ratio: Healthy 0.869 ± 0.39 Infected tissue 0.909 ± 0.16	MSSA 0.12 mg/l	Calculation based on literature data	Cmax/MIC = 10–18	Moxifloxacin reaches high and equal free, protein-unbound concentrations in the interstitial fluid of normal and infected subcutaneous tissue. Results support the previously observed clinical efficacy of moxifloxacin in the treatment of uncomplicated SSI.
Bielecka-Grzela and Klimowicz (2003), 2005 healthy volunteers	400 mg ofloxacin p.o. single dose	Skin	AUCtissue/tplasma = 0.537 ± 0.139				Maximum concentration in microdialysates was significantly lower than in plasma. The kinetics of free, protein-unbound fraction of ofloxacin into skin using cannot be predicted by the calculations based on the plasma drug concentration.
Islinger et al. (2004), 2004 healthy volunteers	320 mg gemifloxacin p.o.	Skeletal muscle subcutaneous adipose tissue	fAUC ratio muscle = 1.7 fAUC ratio adipose = 2.4	*Streptococcus pyogenes* and *Staphylococcus aureus*	Calculation based on literature data	AUC/MIC > 100 h	Gemifloxacin penetrates skeletal muscle and subcutaneous adipose tissues well. Based on PK/PD calculations, gemifloxacin might be a useful therapeutic option for the treatment of soft tissue infections.

fAUC – free area under the concentration-time curve; tAUC - total area under the concentration-time curve MIC - minimum inhibitory concentration; p.o. – *per os;* PD – pharmacodynamics; PK –pharmacokinetics; C_{max} - peak concentration; MDR-TB - multi-drug resistant tuberculosis; MRSA - Methicillin resistant *Staphylococcus aureus;* MSSA - Methicillin sensitive *Staphylococcus aureus;* p.o. - *per os*; CFU - colony forming units; SSI - surgical site infection.

Table 2
Physiochemical and pharmacokinetic properties of selected fluoroquinolones.

Drug name	Bioavailability (%)[a]	Protein binding (%)[a]	Excreted fraction (%)[b]	Metabolised fraction (%)[b]	Half-life (hours)[a]	pKa[b]	logP[b]	VD (l/kg)
Ciprofloxacin	70	30	40–50	15	3.5	6.09	0.28	1.74–5.0 l/kg[d]
Levofloxacin	99	31	87	Limited	6.9	5.45	2.1	1.1 l/kg[c]
Moxifloxacin	86	47	Urine – 20 Feces - 25	52	12.1	5.69	2.9	1.7–2.7 l/kg
Gemifloxacin	71	60–70	Urine - 36 ± 9.3 Feces - 61 ± 9.5	< 10%	8.0	5.53	2.3	1.66–12.12 l/kg
Ofloxacin	98	32	Urine - 65–80 Feces - 4–8	< 10%	9.0	5.45	−0.39	1-1.5 l/kg

Data sources:
[a] Lemke and Williams (2008).
[b] Wishart et al. (2018).
[c] Fish and Chow (1997).
[d] Vance-Bryan et al. (1990).

clinical studies involved experiments in mice, rats, rabbits, chinchillas and dogs (Gao et al., 2007; Kumar et al., 2011; Sawchuk et al., 2005). In clinical setting, for soft tissues and the skin, the procedure is easily managed by any health professional as no special skills are necessary to insert a microdialysis probe. The insertion is no more painful or invasive than the placement of an intravenous catheter (Chaurasia et al., 2007). The probes are usually left *in situ* for several hours, but they can be left in place for up to several weeks. Microdialysis studies do not require the patients to confine to bed-rest. Studies of metabolism and lipolysis in subcutaneous adipose tissue were performed on people during exercise using flexible microdialysis probes (de Lange et al., 2000). The experimental procedure is well tolerated by the patients and no adverse events were reported in assessed studies employing microdialysis in clinical setting.

3. Pharmacokinetics (PK) and pharmacodynamics (PD) of fluoroquinolones

Interpretation of tissue PK requires understanding of antibacterial pharmacodynamics, as the correlation of tissue derived PK with pharmacodynamics offers a description of the time course of effect intensity in response to different drug concentration. Three main measures are used to link drug exposure with bacterial killing (Agwuh and MacGowan, 2006; Ambrose et al., 2007; Craig, 1998, 2001): the fraction of the dosing interval that the concentration of unbound drug is greater than the MIC (ft% > MIC); the ratio of the area under the drug concentration time curve to the MIC (fAUC/MIC) and the ratio of the peak drug concentration during a dosing interval to the MIC (fCmax/MIC). PK/PD markers of efficacy are related to the mechanism of bacterial killing (Jacobs, 2001). Despite the large characteristic number of classes of antimicrobial agents, patterns of antimicrobial activity fall into one of two major patterns: time-dependent activity and concentration-dependent killing (Ambrose et al., 2007; Jacobs, 2001).

Fluoroquinolones kill bacteria in a concentration-dependent manner (Sabo et al., 2015). In general, the minimum bactericidal concentrations are similar to MICs for most bacteria, and there are little differences in the MICs with the increase in the inoculum size. As stated before, for the optimal clinical outcome, high concentrations at the site of the infection are critical for fluoroquinolones (Drusano et al., 2004). Therefore, the peak concentration (C_{max}) and AUC are the main pharmacokinetic parameters taken into calculation when determining efficacy indices for fluoroquinolones (Mueller et al., 2004). Studies proposed Cmax/MIC ratio > 10 (Drusano et al., 2004), is the best predictor of clinical outcome for fluoroquinolones. At lower doses, producing C_{max}/MIC ratios < 10, the AUC/MIC ratio appears to be most closely linked to outcome. Research showed that AUC/MIC value > 25 might be sufficient in less severe infections, but a value of > 100 h appeared necessary for severe infections and/or immune-compromised hosts (Sabo et al., 2015). Retrospective study in patients with *P. aeruginosa* bacteriaemia, showed that the predicted probability of cure was ≥90% when Cmax/MIC was at least 8 (Zelenitsky et al., 2003b). The AUC/MIC of 125 h established by Forrest et al. in the study of pharmacodynamics of ciprofloxacin in critically ill is widely accepted as a target PK/PD value for fluoroquinolones (Forrest et al., 1993), as it was shown to correlate best with rapid bacteriologic and clinical responses.

Some drugs, including fluoroquinolones, exhibit persistent, post-antibiotic effects – inhibition of bacterial growth following short exposure to antibiotics. With involvement of host defense factors, the duration of growth inhibition is usually longer *in vivo* than *in vitro*, which enables longer dosing intervals (Gudmundsson et al., 1986; Vogelman et al., 1988).

4. Fluoroquinolones tissue pharmacokinetics in clinical microdialysis studies

Table 1 lists studies employing microdialysis to the investigation of fluoroquinolone tissue PK in clinical setting, with emphasis on tissue examined, patient characteristics, tissue penetration, PK/PD assessment of tissue concentrations (where available) as well as the main conclusions of examined studies (Bellmann et al., 2004; Bielecka-Grzela and Klimowicz, 2003, 2005; Brunner et al., 1999; Brunner et al., 2002; Burkhardt et al., 2006; Hollenstein et al., 2001; Hutschala et al., 2008; Hutschala et al., 2005; Islinger et al., 2004; Joukhadar et al., 2005; Joukhadar et al., 2003a; Joukhadar et al., 2003b; Kempker et al., 2015; Muller et al., 1999a; Muller et al., 1999b; Schuck et al., 2005; Zeitlinger et al., 2003a; Zeitlinger et al., 2007). In all studies, individual recovery values were used to calculate the absolute concentrations of fluoroquinolones in examined tissues. Recoveries in one *ex vivo* study were determined through zero net flux calibration method (Kempker et al., 2015), all other studies used *in vivo* retrodyalisis. Table 2 summarises physio-chemical and pharmacokinetic properties of fluoroquinolones covered in clinical microdialysis studies. Majority of fluoroquinolones have a molecular mass of around 300 Da, are moderately lipophilic, with the exception of ciprofloxacin, and are 20 to 40% protein bound (Leveque and Jehl, 2009). With most agents being uncharged at the physiological pH, fluoroquinolones distribute easily in peripheral tissues with tissue concentrations often higher than the concurrent serum levels. Most fluoroquinolones are excreted renaly and reach high urinary levels. Moxifloxacin undergoes hepatic metabolism and is eliminated primarily in bile (Zhanel et al., 2006).

4.1. Ciprofloxacin

Despite its hydrophilic properties, ciprofloxacin is well absorbed after oral administration implying an involvement of carrier-mediated transport in their membrane transport process (Arakawa et al., 2012). Ciprofloxacin has been implicated to be a substrate for different

transporters including, but not limited to, P-glycoprotein (P-gp or MDR-1, well known efflux pump expressed in intestinal epithelium, liver cells, proximal tubules and blood-brain barrier (Cordon-Cardo et al., 1990)), Breast Cancer Resistance Protein (BCRP, also known as MXR or ABCG2, an efflux drug transporter located at the at the apical side of enterocytes, hepatocytes and proximal renal cells and organic anion transporting polypeptide (OATP, transporters expressed in liver and many other tissues on basolateral and apical membranes) (Arakawa et al., 2012). Influx transporters such as OATP1A5 (Arakawa et al., 2012) and OATP1A2 (Maeda et al., 2007) are partially responsible for the intestinal absorption of ciprofloxacin. There is still no consensus whether ciprofloxacin is a substrate to P-gp efflux or not. While certain authors claim that P-gp has only a minor contribution to the eflux of ciprofloxacin (Ong et al., 2013), other found that polimorfism in the genes encoding P-gp causes changes in ciprofloxacin clearance (Gorski et al., 2005). On the other hand, BCRP is implicated as transporter that limits ciprofloxacin oral absorption and its increases renal secretion, leading to high urinary concentrations of ciprofloxacin (Haslam et al., 2011; Merino et al., 2006). It was also found that passive and active transport contribute to the uptake of ciprofloxacin through the pulmonary epithelial cells, but due to the low permeation of ciprofloxacin through the secretory pathway, high doses are required to ensure that sufficient concentrations of ciprofloxacin are attained in respiratory tract (Ong et al., 2013). Taking all this into account, studies examining tissue penetration of ciprofloxacin are necessary to clarify the degree to which ciprofloxacin distributes to the tissues of interest. Microdialysis has been employed to measure ciprofloxacin concentrations in muscle and subcutaneous adipose tissues of patients and healthy volunteers. Ciprofloxacin penetrates in the muscle ISF well (fAUC ratio 0.92 to 1.33), with interstitial concentrations corresponding to free plasma concentrations (Brunner et al., 1999; Schuck et al., 2005). Ciprofloxacin penetration in the skin ISF is somewhat lower, with only 0.55 of total plasma concentrations (Bielecka-Grzela and Klimowicz, 2005), therefore complete equilibrium does not ensue between free plasma concentrations and those in skin ISF as around a 30% of total plasma ciprofloxacin is protein bound. In subcutaneous adipose tissue of healthy volunteers, consistent results were reported over several studies with AUC in tissues corresponding to 80–90% of free plasma levels (Brunner et al., 1999; Joukhadar et al., 2001b; Muller et al., 1999a). Microdialysis was employed to compare the penetration of ciprofloxacin into tissue ISF after parenteral and oral administration. Tissue pharmacokinetics was identical after these two means of administration. Brunner et al. (2002) also performed the simulations using *in vitro* models of infection and the ciprofloxacin kill curves indicated comparable pharmacodynamics after 12 h of simulation for ciprofloxacin given at 400 mg i.v. and 500 mg p.o., confirming that both formulations are equivalent in bacteriological effect and not only from a pharmacokinetic point of view.

Factors affecting tissue distribution of ciprofloxacin were elucidated through means of microdialysis. Local inflammation has no significant effect ciprofloxacin distribution. Distribution ratios of ciprofloxacin were similar in infected and non-infected subcutaneous adipose tissue in patients with non-insulin dependent diabetes (Muller et al., 1999a). However, in patients with peripheral occlusive artery disease, ciprofloxacin concentrations were reduced significantly in ischemic lesions compared to the reference tissue. This effect of microvascular occlusion was reversed following percutaneous transluminal angioplasty (Joukhadar et al., 2001b). Before angioplasty area under concentration-time curve values for ciprofloxacin were significantly lower in ischemic tissue (median, 7, range, 3.5–13.0) compared to healthy tissue (median 11.3, range, 3.4–19.0). After angioplasty AUC values were identical in both tissues. *In vitro* simulation based on *in vivo* tissue concentration data indicated that this difference is also reflected in antimicrobial effect. Therefore, improvement of arterial blood flow enhances ciprofloxacin distribution into ISF (Joukhadar et al., 2001b). Similarly, increase of microcirculatory blood flow, which was achieved by warming the examined area in healthy volunteers, and verified by laser Doppler flowmetry, enhanced penetration of ciprofloxacin in subcutaneous adipose tissue of healthy volunteers (Joukhadar et al., 2005). These findings go in favor of views that the reduction of blood flow in soft tissues diminishes the rate of tissue penetration of antimicrobial agents. This is of particular importance in critically ill patients where vasopressor administration can have a marked effect on the antibiotic tissue penetration (Joukhadar et al., 2003a). Impaired blood flow, due to reduced tissue perfusion as well as an impaired endothelium-dependent vasodilation, and reduced relative decrease of capillary surface area was a limiting factor for ciprofloxacin penetration into subcutaneous adipose tissue in obese subjects (Hollenstein et al., 2001). Interstitial concentration did not differ significantly between obese and paired control subjects with physiological weight following actual body weight adjusted doses of ciprofloxacin i.v. in (2.85 mg/kg), but penetration ratio was significantly lower in obese subjects (0.4) compared to lean subjects (0.8) (Hollenstein et al., 2001). In order to achieve the same tissue concentrations, doses in obese subjects need to be based on actual body weight and not ideal body weight, leading to significantly higher plasma levels which poses a risk for adverse effects. With standard dosing, due to impaired tissue penetration, ciprofloxacin concentrations at the site of effect may be sub-inhibitory despite effective concentrations attained in serum (Brunner et al., 2002).

4.2. Levofloxacin

Levofloxacin tissue PK has been studied in interstitial tissue of muscle, subcutaneous adipose tissue and lung tissue in healthy volunteers, as well as patients undergoing surgery, diabetics and patients with sepsis. Microdialysis is a suitable method for sampling levofloxacin from the ISF in clinical setting, with the consistency for drug recoveries and similar PK profiles recorded in several studies. Levofloxacin is a second generation fluoroquinolone with almost 100% bioavailability, and is mostly excreted unchanged through urine (Table 2). After oral administration levofloxacin is readily absorbed, as it has a high solubility and permeability. The OATP1A2 influx transporter has been implicated in the levofloxacin uptake (Maeda et al., 2007), while based on *in vitro* studies, basolateral-to-apical flux of levofloxacin is mediated by P-gp (Naruhashi et al., 2001; Yamaguchi et al., 2000). Elimination of levofloxacin occurs primarily *via* passive renal clearance (Fish and Chow, 1997; Turnidge, 1999). Understanding of transport mechanisms involved in levofloxacin absorption, distribution and elimination helps to comprehend its tissue pharmacokinetics. Next chapters describe the pharmacokinetics of levofloxacin in different patient populations determined by microdialysis.

4.2.1. Patients with sepsis

Higher AUC in muscle than in adipose tissue, similar to plasma values, was measured in healthy volunteers (Zeitlinger et al., 2003a), while in patients with sepsis, tissue/plasma ratio for free levofloxacin, expressed as fAUCtissue/fAUC plasma was 0.85. This tissue penetration in septic patients was reported to be higher than previously registered for beta-lactams and was explained by the accumulation of levofloxacin in leukocytes and other cells, which act as drug depots. Zeitlinger et al. (2003a) measured levofloxacin concentrations in plasma and skeletal muscles of seven patients with sepsis after 500 mg infusion and applied *in vitro* modeling to simulate bacterial killing at the site of infection with respect to concentration-*versus*-time tissue profiles. Despite good tissue penetration of levofloxacin, significant interindividual differences in bacterial killing rates were identified in an *in vitro* model of for *P. aeuruginosa* infection with MIC = 2μg/ml, using muscle PK profiles, while *in vitro* modeling with plasma concentrations demonstrated excellent levofloxacin activity. Study concluded that higher levofloxacin dose may be necessary in septic patients with infections caused by difficult to treat pathogens such as *P. aeruginosa*. This finding also supports the notion that plasma concentrations may be an imprecise

surrogate for tissue concentrations in septic patients. In addition to the dramatically varied PK in these patients, infection by less susceptible bacteria is also common in this population, further reducing the chances of achieving PK/PD thresholds necessary for therapeutic success. Data describing sub-therapeutic concentrations in tissues, particularly in patients with septic shock being treated with vasopressors, exists for various antibiotics including cefpirome, fosfomycin and piperacillin (Joukhadar et al., 2001a; Joukhadar et al., 2002; Roberts et al., 2009; Roberts et al., 2011; Sauermann et al., 2005; Steiner et al., 2004; Zeitlinger et al., 2003b). Severe sepsis and septic shock alter pharmacokinetics of antibacterials due to significant fluid requirements, altered circulation, organ perfusion and hypoalbuminaemia. Especially for hydrophilic drugs, administration of large volumes of resuscitation fluids, increases volume of distribution which is augmented by concomitant hypoalbuminemia leading to lower tissue concentrations (Roberts et al., 2011). Lipophilic antibiotics (such as fluoroquinolones, macrolides, tigecycline, and lincosamides) (Muller et al., 1996) suffer lesser volume of distribution alterations, but may develop altered drug clearances. Both reduced renal clearance due to changes in kidney perfusion and augmented renal clearance owing to hyper dynamic circulatory state and significant fluid requirements as well as hypoalbuminemia and higher unbound concentration in plasma available for renal elimination can be expected. This may result in the impaired transfer of antibiotics from plasma to infection focus despite sufficient concentrations attained in serum. The use of microdialysis for TDM may help overcome the complexity of highly variable pharmacokinetic alterations in septic patients.

4.2.2. Patients with soft tissue infections

Previously mentioned findings (Muller et al., 1999a) that local inflammation appears to have minimal impact on penetration of fluoroquinolones were confirmed by Bellmann et al. (2004). However, that study also noted high interindividual variability in tissue penetration was high, as indicated by a coefficient of variation of approximately 82% for AUC(tissue)/AUC(plasma) ratios (Bellmann et al., 2004). This might be explained by patients not being preselected prior to inclusion into the study, as study examined patients with acute and chronic soft tissue infections, patients with and without occlusive artery disease, diabetics and non-diabetics and the relatively small sample size precluded any subgroup analysis. Nonetheless, authors concluded that levofloxacin exhibited excellent tissue penetration which was not affected by inflammation, while clinical and microbial failure in certain patients can be explained through differences in individual tissue penetration (Bellmann et al., 2004). Treatment resistant patients with severe soft tissue infections may benefit from determination of levofloxacin tissue PK.

4.2.3. Levofloxacin concentrations in lung ISF

Levofloxacin concentration has been measured in interstitial fluid of lungs. At the moment, *in vivo* microdialysis of the human lung is limited to patients undergoing open-chest surgery. The pharmacokinetic profiles derived from the studies in patients undergoing elective lung surgery (Zeitlinger et al., 2007) are in excellent agreement with data derived from patients undergoing coronary artery bypass grafting (CABG) (Hutschala et al., 2005). Results of the levofloxacin pharmacokinetic studies in lung tissue obtained by microdialysis, are highly reproducible irrespective of the patient population. Insertion of microdialysis probes in lung tissue did not cause any difficulties, and adverse events or clinical complications related to the microdialysis procedure were not observed in the reviewed studies. Microdialysis is safe and feasible method for measuring lung concentration profile for fluoroquinolones. A handful of studies in animals and humans indicated that pharmacokinetic data derived from ISF of peripheral tissues can be used to describe the lung PK for the class of beta-lactam antibiotics (Dahyot et al., 2006; De La Pena et al., 2001; Liu et al., 2005; Tomaselli et al., 2003; Tomaselli et al., 2004; Zeitlinger et al., 2005), but this is not applicable to fluoroquinolones. Zeitlinger et al. observed about 1.5 to 2-fold higher $AUCs_{0-\infty}$ for the ISF of muscle and adipose tissue of healthy volunteers compared with lung AUC measured in patients undergoing elective lung surgery, despite no differences in plasma concentration profiles (Zeitlinger et al., 2007). As no dissimilarities were noted in peak muscle, adipose tissue and lung concentrations, these differences in $AUCs_{0-\infty}$ were attributed to the higher clearance of levofloxacin from lung tissue compared with muscle and adipose tissue, confirmed with shorter $t_{1/2}$ in the lung in comparison to peripheral tissues. This might be the consequence of differences in capillary blood flow between lung and peripheral soft tissues (Zeitlinger et al., 2007).

In patients undergoing CABG, the $fAUC_{lung}/AUC_{total}$ in plasma was around 0.6, with small interindividual penetration differences compared to that demonstrated in the muscle tissue of sepsis patients and patients with soft tissue infections (Hutschala et al., 2005). The same team also examined the effect of perioperative atelectasis on levofloxacin lung penetration (Hutschala et al., 2008). The CABG with cardiopulmonary bypass (CPB) served as a model of atelectasis while the control group were patients undergoing the off-pump coronary artery bypass grafting (OPCAB)-technique. They observed significant differences between groups in peak concentration in lung tissue, AUCs0−∞ as well as in AUC ratio and AUCtissue/MIC. Penetration of levofloxacin and AUC/MIC ratio into nondependent lung tissue was significantly higher when OPCAB-technique was used. We must mention that the formation of atelectasis followed by ventilation/perfusion mismatch can be found in a myriad of pulmonary diseases. Atelectasis formation influenced tissue distribution of levofloxacin, and the patients with atelectasis showed significantly lower tissue concentrations in the non-dependent parts of the lung, where measurements were performed. Levofloxacin in usual dose of 500 mg was not associated with sufficient lung tissue concentrations necessary to be effective against *Klebsiella* species in patients undergoing CABG with CPB, and tissue concentrations of levofloxacin were insufficient to treat postoperative infections caused by *P. aeruginosa* in both groups.

According to the studies mentioned above, for majority of gram positive and negative pathogens frequently causing pneumonia, PK/PD calculations based on a PK/PD target value of 30–40 h indicated than optimal bacterial killing can be achieved by levofloxacin. However, the fAUC/MIC ratio revealed that levofloxacin used in the 500 mg dose is borderline sufficient for the treatment of nosocomial pneumonia caused by *Klebsiella pneumoniae* (MIC = 2) and insufficient for the treatment of pneumonia caused by *Pseudomonas aeruginosa*. All studies were consistent in PK/PD analysis to conclude that levofloxacin in a usual dose cannot be recommended for infections caused by *Pseudomonas Auerginosa*, as the MIC of 8 exceeded pulmonary fluid concentrations of levofloxacin by far.

Recent study employed microdialysis technique to study levofloxacin penetration into cavitary tissue in patients with MDR-TB (Kempker et al., 2015). The concentrations of levofloxacin were measured in plasma, whole tissue homogenate and in tissue samples using *ex-vivo* microdialysis and were found to be in a significant correlation. Optimizing serum concentrations therefore helps ensure optimal cavitary levofloxacin concentrations. In majority of patients (64%) levofloxacin had excellent penetration into cavitary lesions with fAUC ratio of 1.33. This high penetration into cavitary lesion compared to lung tissue penetration measured in other studies, might be the consequence of long period of administration of levofloxacin with an average of 313 days and higher dose (750 mg and 1000 mg depending on patient's weight) administered than in other studies. The authors suggested that as a correlation has been found between dose administered (mg per kg) and serum levofloxacin levels, weight-based dosing may represent a way to optimize serum concentrations.

The relevance of antibiotic concentration in the ISF of the lung is still a matter of debate. Many pathogens enter the respiratory system *via* the bronchial pathway with an infection occurring inside the alveolar spaces. Several authors have shown that fluoroquinolone

concentrations in the epithelial lining fluid (ELF) are substantially higher than the plasma levels (Cazzola et al., 2002; Chierakul et al., 2001; Schuler et al., 1997; Yamamoto et al., 2002).

Transporters relevant to distribution of antibiotics in the pulmonary tract include P-gp, organic cation transporters (OCTs) and organic anion transporters (OATs) and the organic anion-transporting polypeptides (OATPs) (Arakawa et al., 2012). Levofloxacin is known substrate to P-gp mediated efflux, and excretion through P-gp could cause higher epithelial lining fluid levels than those attained in plasma. Furthermore, high concentrations in the ELF are also the consequence of delivery to this extracellular site from the intracellular antibiotic reservoirs. Fluoroquinolones are known to accumulate in phagocytic and non phagocitic cells, with intracellular concentrations 10–20 fold higher than those attained in plasma (Drusano et al., 2004). Levofloxacin showed higher concentrations in epithelial lining fluid and alveolar macrophages than in serum, therefore, levofloxacin concentrations measured in lung interstitial fluid might be less important for infections caused by pathogens entering the lung *via* the bronchial system. Also, the intracellular compartment is not accessible to microdialysis, and for respiratory tract infections caused by intracellular bacteria, such as *Legionella* spp. and *Chlamydia pneumoniae*, the ability of fluoroquinolones to concentrate and retain activity intracellularly may be more important than the ISF concentrations. However, the common bacteria causing acute respiratory tract infections are extracellular pathogens and even though the infection starts in alveolar space, after parenchymal consolidation, pneumonia takes place mainly in the interstitium of the lung. Therefore, PK/PD calculations based only on concentrations obtained from ELF may overestimate fluoroquinolone efficacy in cases of severe pneumonia. Also, for certain pathogens, even ELF concentrations may be unsatisfactory. In a murine *Pseudomonas aeruginosa* pneumonia model, with a 750-mg levofloxacin dose, target attainment rates were unsatisfactory at the MIC of 4 mg/l (Louie et al., 2009). Authors concluded that levofloxacin cannot be recommended as a single agent for treatment of *P. Aueruginosa* pneumonia due to such low exposure rates.

Levofloxacin exhibited excellent lung tissue penetration with almost complete equilibrium between free plasma concentrations and target tissues, however, large interindividual differences in tissue PK makes determination of free levofloxacin concentrations with microdialysis a useful step in treatment optimization in respiratory infections.

4.3. Moxifloxacin

Moxifloxacin, fourth-generation synthetic fluoroquinolone is well absorbed after oral administration and is subjected to hepatic metabolism through glucuronide (excreted in urine) and sulfate conjugation (excreted in feces). Moxifloxacin is lipophilic, has low protein binding of and large volume of distribution, which should lead to excellent tissue penetration (Wishart et al., 2018). Data suggests that moxifloxacin interacts with P-gp expressed in Calu-3 lung epithelial cells (Brillault et al., 2009). In the skin, P-gp is expressed on the capillary endothelial cells in venules (Cordon-Cardo et al., 1990), and inflammation and infection cause dynamic changes in P-gp expression (Roberts and Goralski, 2008; Saaby and Brodin, 2017), which may result in differences in drug distribution. Modulation of P-gp activity might therefore influence disposition of moxifloxacin. Moxifloxacin has higher efficacy against Gram positive and anaerobic bacteria compared to levofloxacin and ciprofloxacin. Moxifloxacin is licensed for respiratory infections, skin and skin structure infections, and complicated intra-abdominal infections (Oliphant and Green, 2002). An open, randomized crossover study of moxifloxacin 400 mg p.o. and i.v. in 12 healthy volunteers determined an almost-complete equilibration of the free unbound plasma fraction of moxifloxacin with the interstitial space fluid of subcutaneous adipose tissue and skeletal muscle (Muller et al., 1999b; Oliphant and Green, 2002). Mean fAUC ratios ranged from 0.81 to 0.86. However, these results reflect only drug penetration into physiological compartments, which may not necessarily correspond to pathological situations, *e.g.*, inflamed tissues. Joukhadar et al. (2003b) addressed the ability of moxifloxacin to penetrate into healthy and inflamed subcutaneous adipose tissues in 12 diabetics and non-diabetics with severe soft tissue infections, requiring antimicrobial therapy. In non-diabetic patients, penetration ratio (fAUC) was higher in inflamed tissue (1.2) compared to the healthy tissue (0.5). On the contrary, in diabetics, the ratios of the mean AUC for inflamed tissue (0.5) were lower than in healthy tissue (0.9). Reduced penetration of moxifloxacin into inflamed subcutaneous lesions in diabetics may have been caused by the insufficient vascular supply in the study population. As the mean duration of diabetes was 10 years, some level of micro and macroangiopathies was probably present at the time of the study. Blood flow as a determinant for drug exchange between compartments has also been documented for ciprofloxacin (Joukhadar et al., 2005; Joukhadar et al., 2001b). A PK study of moxifloxacin using total tissue homogenates, also supported the notion that tissue concentrations of moxifloxacin in diabetic patients were lower than those achieved in healthy volunteers (Majcher-Peszynska et al., 2011). On the contrary, a restriction of the blood flow did not play a role on a distribution of moxifloxacin in the interstitial space of normal and infected subcutaneous tissue in patients with spinal cord injury (Burkhardt et al., 2006). Moxifloxacin concentrations were measured in decubitus ulcer which are known to form due to limited vascular supply. Nonetheless, no difference was noted in moxifloxacin tissue concentrations in normal subcutaneous tissue and infected decubitus ulcers. However, in patients with spinal cord injury, a certain degree of vascular impairment in the reference tissue cannot be excluded, making the direct comparison with other studies difficult.

All of the above raises a question, are the moxifloxacin tissue concentration in the infected tissues high enough for effective bacterial killing? Complicated skin and skin structure infections are often polymicrobial, requiring broad-spectrum combination therapy, and with good *in vitro* activity, against gram-positive and gram-negative aerobes and anaerobes including the causative pathogens implied in diabetic foot infections, moxifloxacin may be useful for initial empirical therapy. In healthy volunteers, with the MIC of relevant pathogens at the target sites (0.12), based on interstitial concentrations, Cmax/MIC ratios of 8 were achieved, which corroborates the role of moxifloxacin as a highly effective antimicrobial agent (Muller et al., 1999b). In diabetics, the moxifloxacin concentrations and the AUC in the inflamed lesions were lower than those in the inflamed lesions of nondiabetic patients. PK/PD analysis based on free plasma concentrations and most common bacterial strains causing skin and soft tissue infections (MIC = 0.12–0.25 mg/l) yielded fAUC/MIC ratios between 58 and 121 h and C_{max} close to the mutant prevention concentration. In patients with decubital ulcers, moxifloxacin reached adequate concentrations in normal subcutaneous tissue and infected decubitus ulcer tissue, and free, protein-unbound peak concentrations of moxifloxacin in infected ulcer tissue amounted to 10- to 18-fold of a MIC90 value of 0.12 mg/l (Burkhardt et al., 2006). The PKs of moxifloxacin in plasma and tissue indicate that moxifloxacin qualifies for the management of skin and soft tissue infections (SSI) in both diabetics and non-diabetics. Several randomized clinical trials demonstrated that moxifloxacin is clinically comparable to the piperacillin-tazobactam/ amoxicillin-clavulonic in patients with moderate to severe SSIs (Gyssens et al., 2011; Lipsky et al., 2007). However, moxifloxacin EUCAST sensitivity breakpoint is set at 0.25, therefore it is questionable whether all pathogens classified as susceptible can be successfully eradicated with moxifloxacin. MIC testing is performed in serial dilutions, and with a single step increase (from 0.12 to 0.25 mg/l) two fold higher C_{max} levels are necessary to provide the same exposure. Population pharmacokinetics and target attainment analysis of moxifloxacin in patients with diabetic foot infections noted limited probability of target attainment for formally susceptible Gram-negative pathogens close to EUCAST breakpoint, and concluded that special attention should be given to

patients where rare gram negative pathogens with a moxifloxacin MIC of 0.25 mg/L are isolated (Wicha et al., 2015).

4.4. Other fluoroquinolones

Determination of ofloxacin, a racemic mixture of levofloxacin and dextrofloxacin in skin ISF established that the maximum concentration in cutaneous dialysate was achieved somewhat later than in plasma with a degree of penetration into cutaneous microdialysate of 0.54. Skin penetration of ofloxacin was similar to that of ciprofloxacin (Bielecka-Grzela and Klimowicz, 2003, 2005). Authors also created individual time-concentration curves for theoretical peripheral compartment and concluded that kinetics of free, protein-unbound fraction of ofloxacin cannot be predicted using the theoretical calculations based on the plasma drug concentration alone (Bielecka-Grzela and Klimowicz, 2005).

Penetration of gemifloxacin, a fourth generation fluoroquinolone, into subcutaneous and muscle tissue was examined in healthy volunteers (Islinger et al., 2004). The penetration ratio measured for gemifloxacin was higher than for other fluoroquinolones, especially in adipose tissue. High lipophilicity of gemifloxacin and lower blood perfusion rate of healthy adipose tissue than of muscle tissue resulting and lower clearance could also contribute to higher concentrations attained in the adipose tissue compared to muscle. Also, the calculated ratios of PK/PD indices for pathogens commonly causing soft tissue infections, such as *Streptococcus pyogenes* and *Staphylococcus aureus* using tissue PK data exceed the target breakpoint for efficient dosing of 100 h. Gemifloxacin concentrations in the extracellular space fluid in soft tissues are adequate for effective antimicrobial effect, but this data from healthy volunteers may not necessarily translate to patients with soft tissue infections.

4.5. Selected animal studies employing microdialysis

For certain tissues, where clinical microdialysis was not performed, animal derived data can offer important information of fluoroquinolone penetration. For an example, microdialysis has been used to measure free levofloxacin concentrations in prostatic tissue of rats (Hurtado et al., 2014). Unbound levofloxacin prostate concentrations measured by microdialysis were compared to those in plasma after a 7-mg/kg intravenous bolus dose to Wistar rats. After *i.v.* administration, levofloxacin quickly distributed throughout the body, as evidenced by the fast exponential decay in the plasma concentrations, reaching unbound prostate tissue concentrations of 78% of unbound plasma levels. Prior studies reported levofloxacin concentration ratio of ≈3 for prostate tissue:serum using bioptates of prostate tissue (Drusano et al., 2000; Hurtado et al., 2014; Szerkus et al., 2016). These findings demonstrate how the measurements in whole tissue homogenates can lead to overestimation of the target site concentrations and clinical efficacy. Findings from a microdialysis study suggested that active efflux pump plays a role in the prostate penetration of quinolones, assuming that the distribution of levofloxacin into prostate is governed by diffusion (Hurtado et al., 2014).

Microdialysis has also been employed to measure free levofloxacin concertation in rat pancreas after oral and intravenous administration, mimicking the human dose of 400 mg/day (Liu et al., 2014). Levofloxacin was equally distributed into ISF of rat pancreatic tissue with intravenous (AUCpancreas/AUCblood, 0.97 ± 0.02) and oral (AUCpancreas/AUCblood, 0.96 ± 0.03) route of administration. Similarly, the simultaneous determination of unbound levofloxacin in rat blood and bile suggested that there was rapid exchange and equilibration between the blood and hepatobiliary systems, and implied that levofloxacin undergoes hepatobiliary excretion (Cheng et al., 2002). The AUC of unbound pefloxacin in bile was significantly greater than that in blood, suggesting active biliary excretion of pefloxacin (Tsai, 2001).

Brain microdialysis studies in rats provided the information that certain members of fluoroquinolone class have restricted penetration into brain ISF, despite attaining sufficient concentrations in the cerebrospinal fluid (CSF). In humans, the easiest way to study antibiotic penetration into CNS is to measure the concentration in CSF from lumbar puncture or external ventricular drain. Concentrations in the CSF are considered a good surrogate for brain target site concentrations. However, there are differences between the blood-brain-barrier and blood-CSF barrier, which result in differences of drug distribution between CSF and brain ISF. Penetration ratios, expressed as AUC_{brain}/AUC_{blood} were 0.036 and 0.05 for pefloxacin and norfloxacin respectively (Marchand et al., 2003; Marchand et al., 2006; Tsai, 2001). Penetration of norfloxacin was independent of dose administered. Steady-state brain ISF for norfloxacin, ofloxacin, fleroxacin, and pefloxacin measured by brain microdialysis were 7–30 times lower than the free serum concentrations. CSF concentrations of the quinolones were twice as high as the brain ISF concentrations, except for norfloxacin. For sparfloxacin, study in mdr1a (−/−) mice and wild-type mice microdialysis data implicated that the absence of P-glycoprotein increases brain disposition of sparfloxacin. No difference in plasma concentration was observed between two groups of animals, while the concentrations in brain ISF were 4-fold higher in the mdr1a (−/−) mice (de Lange et al., 2000). Passive diffusion, followed by efflux across the blood-brain barrier, has been suggested as the predominant pathway for quinolone elimination from the cerebrospinal fluid (Ooie et al., 1997).

5. Conclusion

Microdialysis is a very useful technique for better understanding fluoroquinolone distribution and penetration, and offers unique opportunity to model the fluoroquinolone efficacy based on actual tissue pharmacokinetic data. Microdialysis is state-of-the art technique for sampling and measuring interstitial drug concertation and subsequent dosage optimization of anti-infective agents, including fluoroquinolones. Results of the reviewed studies offer evidence that blood flow is a limiting factor for fluoroquinolone penetration into target tissues. In obese patients, penetration rates were found to be lower than in healthy volunteers. Large interindividual differences for levofloxacin penetration into muscle and adipose tissue were found, while the lung penetration was similar across several studies and patient populations. Microdialysis supports the use of moxifloxacin in skin and soft tissue infection. Tissue concentrations of fluoroquinolones cannot be extrapolated solely from plasma data.

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