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1 **Lung Microdialysis Study of Levofloxacin in Rats following**
2 **Intravenous Infusion at Steady-State**

3

4 **Running Title: Microdialysis study of levofloxacin in lung**

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1 **ABSTRACT:**

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3 A microdialysis distribution study of levofloxacin in the lung and muscle of rats was
4 conducted from a single point determination after intravenous infusion at steady-state.

5 This approach was presented as an interesting alternative to investigate antibiotics tissue
6 distribution.

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1 Several different methodological approaches are available to assess the distribution of
2 antibiotics in the lung of animals or patients (12). It is now recognized that drug
3 concentrations in whole tissue homogenates are difficult to interpret and therefore not
4 informative (18). In human the most popular experimental approach to characterize
5 antibiotics distribution in lungs relies on bronchoalveolar lavages (BAL) (3, 4, 5) and
6 micro-BAL was recently proposed (2). Microdialysis is an appealing technique that was
7 increasingly used over the last years to investigate antibiotics distribution in the
8 extracellular fluid (ECF) of various tissues including lung, both in rats and humans (6,
9 11, 15, 17, 19, 23). One of the major advantages of microdialysis over BAL or micro-
10 BAL is that it allows multiple determinations in the same subject and therefore full
11 description of the drug concentrations versus time profile following its administration.
12 However in human lung microdialysis studies are limited to patients with elective
13 thoracic surgery and in rats they require maintenance of the animal anaesthetized with
14 open chest surgery (6, 17). As a consequence for antibiotics with long elimination half-
15 lives, it may be difficult to maintain study duration for a period of time long enough to
16 properly characterize major pharmacokinetic parameters such as total drug concentrations
17 versus time area under curves (AUCs). Comparison of total unbound AUCs in lung ECF
18 and plasma may provide important information such as indirect evidence for the
19 involvement of active efflux transport systems (7). These systems, including P-
20 glycoprotein (P-gp), are present in brain and are responsible for lower unbound AUCs in
21 brain ECF than in plasma as previously demonstrated with several anti-infectious drugs
22 using microdialysis (8, 10, 13, 16). The presence of P-gp in lung is suspected (9, 14, 20,
23 21) and therefore potentially responsible for restricted drug tissue distribution, although

1 this has never been documented, at least to our knowledge. In order to do that it should be
2 possible to compare total unbound AUCs in lung tissue ECF and plasma following single
3 dose administration, but interestingly comparing unbound drug concentrations following
4 intravenous infusion at steady-state would provide exactly the same information (7). The
5 advantage of steady-state conditions is to require a single concentration determination in
6 plasma and tissue, which is much more simple and probably more accurate than
7 determining AUCs after single dose administration. The feasibility and optimization of
8 such an alternative approach is now being tested using levofloxacin (LVX) as a
9 representative antibiotic.

10

11 Experiments were done in accordance with the Principles of Laboratory Animals Care
12 (NIH Publication #85-23, revised 1985) using male Sprague Dawley rats (Janvier
13 Laboratories, Le Genest-St-Isle, France), weighing 316 ± 49 g. The day before
14 experiments (D-1), between 9:00 pm and 11:00 am, rats were anesthetized with air-
15 isoflurane mixture and equipped with a vein femoral catheter for LVX administration and
16 with microdialysis CMA/20 probes (polycarbonate, cutoff 20000 Da, membrane length
17 10 mm, CMA microdialysis, Phymep, Paris, France) in blood and muscle for drug
18 concentrations determinations, as previously described (17). After rats wake up and six to
19 seven hours post surgery, infusion of a commercial solution of LVX (5 mg.mL^{-1} , Sanofi
20 Aventis, Paris, France) was started at a rate of 1.0 mg.h^{-1} , maintained overnight to reach
21 steady-state and until the end of experiment. On the morning of day zero (D0) rats were
22 anesthetized, tracheotomized, mechanically ventilated and torachotomized for lung
23 microdialysis probe insertion (LMP 5.35.35, polyether sulfone, cut off: 6000 Da,

1 membrane length: 10mm, outer diameter: 0.6 mm, Microbiotech, Stockholm, Sweden) as
2 previously described (17). Probes recoveries were estimated by the retrodialysis by
3 calibrator method. A solution of ciprofloxacin (CIP) ($2 \mu\text{g.mL}^{-1}$) was perfused into the
4 three probes at a flow rate of $2 \mu\text{L.min}^{-1}$ for 15 min. A flow rate equal to $0.5 \mu\text{L.min}^{-1}$
5 was then maintained for 60 min before starting dialysates collection, and until the end of
6 the experiment. Dialysates were collected from blood, muscle and lung probes every 45
7 min over 270 min, corresponding to 6 samples. The relative recovery by loss of the
8 calibrator (CIP) was calculated for each interval of collect according to the following
9 equation: $RL_{\text{CIP}} = (\text{Cin} - \text{Cout}) / \text{Cin}$, where Cin and Cout correspond to the CIP
10 concentrations in perfusate and in dialysates collected. Actual LVX concentrations were
11 estimated by correcting measured concentrations in dialysates by the recovery by loss of
12 CIP determined during the same interval of collect. For each rat and medium ECF
13 concentrations at steady-state are presented as a mean \pm S.D value derived from the 6
14 consecutive determinations. The *in vitro* recoveries by gain and by loss of CIP and LVX
15 were evaluated from three probes (two CMA 20 probes and one linear probe) during 240
16 min at a flow rate of $1 \mu\text{L.min}^{-1}$ and concentrations equal to $3 \mu\text{g.mL}^{-1}$. *In vivo* recoveries
17 by loss of CIP and LVX were compared in 3 dedicated rats: A, B and C. A mixture of the
18 2 compounds, each at a concentration of $2 \mu\text{g.mL}^{-1}$, was perfused in each probe at a flow
19 rate of $0.5 \mu\text{L.min}^{-1}$ during 270 min and dialysates were collected by fractions every 45
20 min. Simultaneous analyse of LVX and CIP in dialysates were performed by HPLC as
21 previously described (16). Dialysates were injected directly after dilution in phosphate
22 buffer (pH=7) (1/1, vol/vol). Both compounds were analyzed at the same wavelengths:
23 $\lambda_{\text{ex}}=285 \text{ nm}$, $\lambda_{\text{em}}=490 \text{ nm}$. The between-day variability of the assay was characterized

1 each day of the analysis and was respectively less than 20% and 10% at two
2 concentrations levels (0.125 and 1 $\mu\text{g}\cdot\text{mL}^{-1}$).

3

4 Steady-state conditions preclude the retrodialysis by drug method to estimate probes
5 recoveries, and considering that our objective was to conduct an experiment within a
6 limited period of time, the retrodialysis by calibrator method was the best method for
7 that. CIP had been previously validated as an appropriate calibrator for another
8 fluoroquinolone antibiotic, norfloxacin (NOR) (16). It was therefore tested again as a
9 potential calibrator for LVX. *In vitro* recoveries by gain and by loss of CIP and LVX
10 were not statistically different (data not shown). Differences between LVX and CIP *in*
11 *vivo* recoveries by loss in blood, muscle and lung, were always lower than 7%, with only
12 an exception for Rat C in lung (Table 1).

13

14 Individual values of CIP recoveries by loss determined in rats receiving LVX (Rats 1-8)
15 are presented in Table 2. These values were generally consistent with those obtained
16 during the validation phase (Rats A-C), although relatively higher recoveries were
17 occasionally observed especially in blood. Recovery by loss of CIP in lung was 3 to 4
18 folds lower on average than in muscle or blood (Table 2), again consistent with
19 observations made in Rats A-C, as well as with previous experiments (6, 17). These
20 relatively low recoveries in lung are most likely due to the different nature and cut-off of
21 the membrane compared to CMA/20 probes. The between rats variability in lung probes
22 recoveries, with extreme values ranging between $9.2 \pm 3.6\%$ and $31.5 \pm 7.5\%$ was also
23 larger than in other media (Table 2). Altogether these data suggest that CIP may be

1 considered as an appropriate calibrator for LVX, although discrepancies between the
2 estimated recovery by loss of the calibrator and the actual recovery of the tested
3 compound may occasionally occur, especially in lung, possibly leading to outliers values
4 in the estimated LVX concentrations.

5

6 Individual steady-state unbound concentrations of LVX in each medium are presented on
7 Figure 1. The between rats as well as the between medium variability was rather limited
8 and average unbound steady-state concentrations in the various rats and medium
9 compared favorably with only one exception (lung data in Rat 3). Errors bars
10 corresponding to standard deviations associated to these estimated average concentrations
11 (n=6) were most often higher in lung than in other medium, illustrating the greater
12 uncertainty on concentrations estimates in this tissue compared to plasma or muscle. This
13 observation which is again consistent with previous findings (6, 17) is probably a
14 consequence of the lower lung probes recoveries. Because flow rate was already quite
15 low ($0.5 \mu\text{L}\cdot\text{min}^{-1}$), it does not seem possible to substantially increase lung probes
16 recoveries by further reducing the flow. Using different probes with increased recovery
17 should potentially be of greater benefit.

18

19 ECF steady-state unbound tissue to blood concentrations ratios were respectively equal to
20 1.00 ± 0.15 in muscle and 1.06 ± 0.40 in lung, suggesting passive tissue distribution of
21 LVX (7). These values are in partial agreement with data previously obtained in human
22 and published mostly by researchers from the same group (1, 11, 22, 24). The first LVX
23 microdialysis distribution study in human was conducted in the skeletal muscle tissue of

1 patients with sepsis (22). Unbound plasma concentrations were derived from measured
2 total plasma concentrations assuming an average fraction bound equal to 35%. Ratio of
3 the tissue to plasma unbound AUCs from time 0 to 8h (AUC_{0-8}) was used to characterize
4 LVX distribution and was estimated to 0.85. The second study was conducted in
5 subcutaneous adipose tissue using similar procedure, except that AUCs were estimated
6 from time 0 to 10h and plasma protein binding was assumed to be equal to 25% (1).
7 Average tissue to plasma unbound AUCs ratios were again close to unity (1.1 ± 0.6 for
8 healthy and 1.2 ± 1.0 for inflamed subcutaneous adipose tissue). The third article
9 describes the first microdialysis distribution study in lung (11) and lead to the conclusion
10 that LVX penetration in this tissue was lower than in muscle (22) or subcutaneous
11 adipose tissue (1). However in this study unbound AUCs of LVX in lung were compared
12 to total plasma AUCs, with a mean ratio equal to 0.6. Therefore after correcting plasma
13 AUC for protein binding as previously done by these authors, and considering that 30%
14 of LVX is bound in plasma, one would obtain a mean tissue to plasma unbound AUCs
15 ratio equal to 1.9, that is actually higher than the previously reported values close to unity
16 (1, 22). The last study was then conducted to clarify the apparently conflicting data
17 between muscle or subcutaneous tissue and lung (24). Unfortunately distinct subjects
18 were enrolled in the study with muscle and subcutaneous distribution being investigated
19 in healthy volunteers and lung distribution in patients undergoing elective lung surgery.
20 Furthermore unbound tissue concentrations determined by microdialysis were again
21 compared with total plasma concentrations making AUCs ratios interpretation difficult,
22 especially since LVX plasma protein binding may vary between healthy volunteers and
23 patients. But interestingly in this last study total AUCs were used to characterize LVX

1 tissue distribution. Unfortunately microdialysate samples were collected over a 8 h period
2 of time, which considering the delayed peak in tissue (between 1h and 3h) and the
3 relatively long elimination half-life of LVX (estimated to 5- 6h in this study), does not
4 allow precise estimation of total AUCs. This study lead to the conclusion that LVX levels
5 in the ECF of soft tissues cannot serve as a surrogate for predicting its pharmacokinetics
6 in lung (24), whereas the results of our study that was conducted in rats but measuring
7 LVX concentrations in both tissues of each animal, suggest the opposite.

8

9 Microdialysis tissue distribution studies following single doses administrations should
10 rely on the comparison between total unbound AUCs in tissue and plasma (7), but as
11 exemplified by this series of studies conducted with LVX this is not always done, and it
12 may be difficult to accurately estimate these total AUCs. On many occasions AUCs
13 between two consecutive administrations at steady-state should be equal to total AUCs
14 after single doses, but they may not be more practical to estimate. Therefore comparison
15 of tissue ECF and plasma unbound concentrations following intravenous infusion at
16 steady-state appears as an interesting alternative to characterize drug tissue distribution,
17 in particular to look for active efflux transport phenomenon. Although a single
18 concentration measurement in tissue and plasma would be enough from a theoretical
19 standpoint, six consecutive series of determinations were conducted for this initial study.
20 Yet this was without major benefit since these individual values were always consistent
21 and the number of determinations should be reduced for the next studies. Yet as opposed
22 to microdialysis studies conducted with multiple drug determinations after single dose
23 administrations, the major limitation of this proposed approach based on single points

1 determination at steady-state, is that it does not provide any information on the tissue rate
2 of distribution, that can be modified in the presence of various patho-physiological
3 conditions such as altered blood flow.

4

5 In conclusion lung microdialysis investigations following drug infusion at steady-state
6 appear as an interesting alternative to the same type of experiments conducted after single
7 dose administration for the characterization of lung distribution of antibiotics.

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1 **TABLE 1.** *In vivo* recoveries by loss of CIP and LVX (mean \pm SD, n=6) perfused as a
 2 Ringer solution (CIP / LVX mixture at 2 $\mu\text{g}\cdot\text{mL}^{-1}$ each) at a flow rate of 0.5 $\mu\text{L}\cdot\text{min}^{-1}$
 3 during 270 min, in the blood, muscle and lung probes of 3 rats (A-C)

| | | <i>In vivo</i> recoveries by loss (%) (n=6) | | |
|--------------|------------|---|----------------|----------------|
| | | Blood | muscle | Lung |
| Rat A | LVX | 43.6 \pm 5.6 | 38.9 \pm 1.7 | 11.4 \pm 3.8 |
| | CIP | 42.9 \pm 5.9 | 35.9 \pm 1.5 | 11.3 \pm 3.8 |
| Rat B | LVX | 48.0 \pm 3.0 | 36.8 \pm 0.7 | 12.8 \pm 3.0 |
| | CIP | 49.1 \pm 3.1 | 36.1 \pm 1.8 | 10.9 \pm 4.0 |
| Rat C | LVX | 51.0 \pm 2.1 | 36.1 \pm 3.9 | 6.9 \pm 2.4 |
| | CIP | 56.6 \pm 2.0 | 39.1 \pm 4.4 | 11.9 \pm 2.4 |

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1 **TABLE 2.** *In vivo* recoveries by loss of CIP (mean \pm SD, n=6) perfused as a Ringer
2 solution (2 $\mu\text{g.mL}^{-1}$, 0.5 $\mu\text{L.min}^{-1}$) in the blood, muscle and lung probes of 8 rats
3 receiving an intravenous infusion of LVX (1.0 mg.h^{-1}) at steady state .

4

| CIP <i>in vivo</i> recoveries by loss (n=6) | | | |
|--|----------------|----------------|-----------------|
| | blood | muscle | lung |
| Rat 1 | 44.6 \pm 3.1 | 45.0 \pm 3.9 | 31.5 \pm 7.5 |
| Rat 2 | 41.0 \pm 3.6 | 38.4 \pm 4.4 | 9.4 \pm 2.5 |
| Rat3 | 32.6 \pm 2.2 | 48.4 \pm 2.9 | 22.8 \pm 10.0 |
| Rat 4 | 74.6 \pm 1.1 | 30.8 \pm 1.4 | 18.8 \pm 3.5 |
| Rat 5 | 38.4 \pm 1.1 | 45.0 \pm 1.4 | 10.4 \pm 3.3 |
| Rat 6 | 46.3 \pm 4.3 | 46.6 \pm 2.4 | 12.1 \pm 8.2 |
| Rat 7 | 73.4 \pm 1.2 | 48.5 \pm 1.7 | 15.9 \pm 3.9 |
| Rat 8 | 73.4 \pm 1.3 | 48.5 \pm 1.8 | 15.9 \pm 3.1 |

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- 1 **FIG. 1.** Mean unbound concentrations of LVX (n=6) in muscle ECF, blood and lung
- 2 ECF, of 8 rats receiving an intravenous infusion of LVX ($1.0 \text{ mg}\cdot\text{h}^{-1}$) at steady state .

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