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Tissue Distribution of [^{14}C]-Lefamulin Into the Urogenital Tract in Rats

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ABSTRACT

Background: Lefamulin is the first semisynthetic pleuromutilin antibiotic for IV and oral use in humans and is currently in phase 3 trials for the treatment of community-acquired bacterial pneumonia (CABP). Lefamulin has been shown to be highly active against bacterial pathogens causing sexually transmitted infections (STIs), including multidrug-resistant strains of *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Mycoplasma genitalium*. To investigate the distribution of lefamulin into the urogenital tract tissues in rats, we combined quantitative whole-body autoradiography (QWBA) with tape transfer microautoradiography (MARG).

Methods: [^{14}C]-lefamulin was administered to nonpigmented male rats as a single IV dose of 30 mg/kg. The tissue distribution was investigated by QWBA and tape-transfer MARG of sagittal planes at 0.5, 6, and 24 h postdose allowing investigation of the distribution of [^{14}C]-lefamulin down to a cellular level. Distribution of radioactivity was determined and quantified using a storage phosphor image analyzing system. Interpretation of MARG results was qualitative and supported by images captured with the associated digital camera.

Results: The analysis of sagittal sections of male rats following 30 mg/kg [^{14}C]-lefamulin revealed high concentrations in certain glandular tissues. Example MARGs of the prostate gland at 6 h are shown in the **Figure 1**. In the prostate gland, the tissue/blood ratio at 6 h calculated for the lumen and the wall was 9.98 and 16.3, respectively.

Conclusion: [^{14}C]-lefamulin in rats showed rapid and homogeneous distribution into the urogenital tract tissues down to a cellular level, with high tissue/blood ratios in relevant STI tissues. Based on these results and the potent *in vitro* activity against the multidrug-resistant bacterial causes of STIs, further assessment of lefamulin for the treatment of STIs is warranted.

INTRODUCTION

- Sexually transmitted infections (STIs) have a major impact on reproductive health worldwide, with more than 1 million STIs acquired every day[1]
- In the United States the most common bacterial causes of STIs are *Chlamydia trachomatis* and *Neisseria gonorrhoeae,* and increases in incidence rates have been reported[2]
- Lefamulin is the first semisynthetic pleuromutilin antibiotic in clinical development for intravenous and oral use in humans; lefamulin inhibits bacterial protein synthesis by binding to the peptidyl transferase center of the 50S ribosomal subunit[3]
- A recently completed phase 3 clinical trial in adults with community-acquired bacterial pneumonia showed noninferiority of lefamulin vs moxifloxacin ± linezolid
- Lefamulin is highly active against bacterial pathogens causing STIs, including multidrug-resistant strains of *N. gonorrhoeae*, *C. trachomatis*, and *Mycoplasma genitalium*[4,5]
- Pharmacokinetic studies have shown rapid lefamulin penetration into the interstitial space of skeletal muscle, subcutaneous adipose tissue and epithelial lining fluid, with exposure levels in the epithelial lining fluid 5.7-fold higher than the free fraction in plasma[6]
- The purpose of this study was to investigate the distribution of lefamulin into the urogenital tract tissues in rats using quantitative whole-body autoradiography (QWBA) with tape transfer microautoradiography (MARG)

METHODS

- [^{14}C]-lefamulin was administered to nonpigmented rats (3 males and 3 females) as a single intravenous dose of 30 mg/kg
- At 0.5, 6, and 24 hours postdose, the tissue distribution of [^{14}C]-lefamulin down to a cellular level was investigated by QWBA and MARG of sagittal planes
- Distribution of radioactivity ([^{14}C]-lefamulin) was determined using a storage phosphor image analyzing system
  - Interpretation of MARG results was qualitative and supported by images captured with the associated digital camera
- Selected samples were prepared for quantitative radiochemical analysis by liquid scintillation counting, and data were expressed as microgram equivalents per gram of original sample weight

RESULTS

Lefamulin Distribution in the Male Urogenital Tract

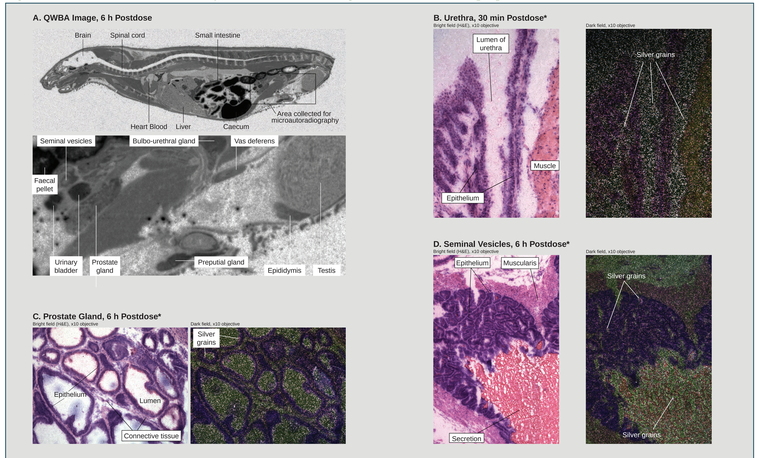
- High concentrations of [^{14}C]-lefamulin were observed in certain glandular tissues (eg, preputial, bulbourethral) and in the urethra, with concentrations similar to those found in the lung **(Table 1)**
- At 30 minutes and 6 hours postdose in the urethra, lefamulin concentrations were similar (22.3 and 24.0 µg [^{14}C]-lefamulin/g tissue, respectively), which corresponded to 6.6 and 36.6 times higher than in the blood at each time point (**Table 1** and **Figure 1**)
  - By 24 hours postdose, lefamulin was nearly cleared from the urethra, with only 0.8 µg [^{14}C]-lefamulin/g tissue detected
- MARG of the prostate gland and seminal vesicles showed higher concentrations of radioactivity associated with the walls (epithelium and mucosa) vs the lumen **(Figure 1)**
  - In the prostate gland, the tissue/blood ratio peaked at 6 hours postdose, with ratios for the wall and the lumen at 19.0 and 10.0, respectively **(Table 1)**
  - The wall of the seminal vesicle also had higher lefamulin concentrations than the lumen (tissue/blood ratio, 6 hours postdose: 16.3 vs 6.5, respectively; **Table 1**)
- Concentrations in several urogenital tract tissues at 24 hours after administration were similar to those observed in the lung at that time **(Table 1)**

Table 1. Concentration of [^{14}C]-Lefamulin in the Urogenital Tract of Male Rats at 0.5, 6, and 24 Hours After Administration

	µg Equivalents*			Tissue: Blood Ratio			Tissue: Lung Ratio		
Male Tissue	0.5 h	6 h	24 h	0.5 h	6 h	24 h	0.5 h	6 h	24 h
Blood	3.4	0.7	BLQ	1.0	1.0	NC	0.1	0.03	NC
Lung	26.5	20.5	0.4	NA	NA	NA	1.0	1.0	1.0
Bulbourethral gland	53.4	13.4	NS	15.8	20.5	NC	2.0	0.7	NC
Preputial gland	NS	15.4	9.8	NC	23.5	NC	NC	0.8	25.9
Prostate gland, whole	11.0	8.4	0.5	3.3	12.8	NC	0.4	0.4	1.3
Prostate gland, lumen	4.6	6.5	0.3	1.4	10.0	NC	0.2	0.3	0.8
Prostate gland, wall	14.4	12.5	0.7	4.3	19.0	NC	0.5	0.6	1.9
Seminal vesicles, whole	8.3	7.6	0.5	2.5	11.6	NC	0.3	0.4	1.2
Seminal vesicles, lumen	2.7	4.2	0.3	0.8	6.5	NC	0.1	0.2	0.8
Seminal vesicles, wall	16.7	10.7	0.6	5.0	16.3	NC	0.6	0.5	1.6
Testis	1.3	2.3	2.0	0.4	3.5	NC	0.1	0.1	5.2
Urethra	22.3	24.0	0.8	6.6	36.6	NC	0.8	1.2	2.1
Urinary bladder, whole	19.5	20.4	0.6	5.8	31.2	NC	0.7	1.0	1.6
Urinary bladder, contents	24.2	35.0	0.9	7.2	53.4	NC	0.9	1.7	2.2
Urinary bladder, wall	8.2	3.4	0.1	2.4	5.1	NC	0.3	0.2	0.2

BLQ=below limit of accurate quantification (<0.062 µg equivalents/g); NA=not applicable; NC=not calculable (tissue is BLQ/NS at this time); NS=no sample, tissue not sectioned.
*Data are µg equivalents of lefamulin per gram tissue

Figure 1. Distribution of Radioactivity in Male Rats Following IV Administration of [^{14}C]-Lefamulin



IV=intravenous; H&E=Haemotoxylin and Eosin; QWBA=quantitative whole-body autoradiography.
*Under bright field conditions, radioactive areas (silver grains) show as small particles and the tissue histology can be viewed. Under dark field conditions, silver grains show up bright white against a dark, false-color background.

Lefamulin Distribution in Female Urogenital Tract

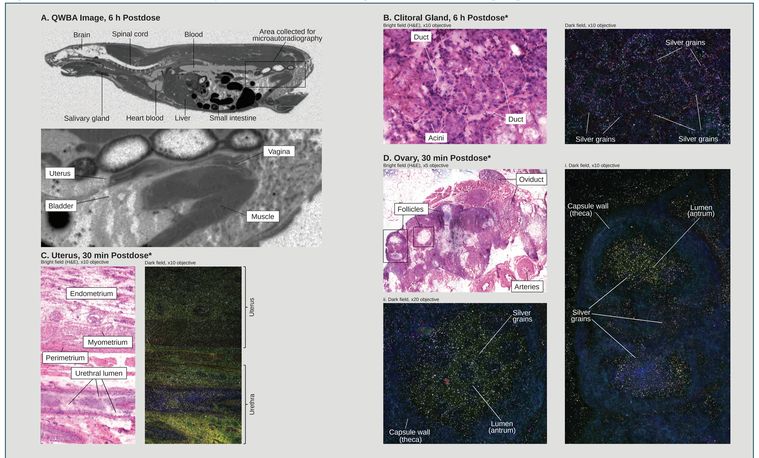
- [^{14}C]-lefamulin distribution was homogenous across the lumen, epithelium, and smooth muscle wall of the vagina
- Highest concentrations of [^{14}C]-lefamulin were observed in the clitoral gland, the uterus, and the urethra **(Table 2)**
- At all time points sampled, the greatest accumulation of [^{14}C]-lefamulin was seen in the clitoral gland, with levels 2- to 3-fold greater than the lung
  - At 6 hours postdose, the radioactivity was most concentrated in the ducts surrounding the acini rather than within these structures **(Figure 2)**
- At 30 minutes postdose, the endometrium (internal uterine mucosa) had similar concentrations of lefamulin compared with the rest of the uterus (30.6 and 31.9 µg [^{14}C]-lefamulin/g tissue, respectively); however, at 6 hours postdose the endometrium had higher concentrations than the uterus (13.4 and 8.4; **Figure 2** and **Table 2**)
- The [^{14}C]-lefamulin detected in the ovary was mainly associated with the follicular lumen **(Figure 2)**

Table 2. Concentration of [^{14}C]-Lefamulin in the Urogenital Tract of Female Rats at 0.5, 6, and 24 Hours After Administration

	µg Equivalents*			Tissue: Blood Ratio			Tissue: Lung Ratio		
Female Tissue	0.5 h	6 h	24 h	0.5 h	6 h	24 h	0.5 h	6 h	24 h
Blood	5.6	1.1	BLQ	1.0	1.0	NC	0.1	0.05	NC
Lung	39.5	22.4	0.8	NA	NA	NA	1.0	1.0	1.0
Clitoral gland	81.3	NS	2.4	14.5	NC	NC	2.1	NC	2.9
Ovary	27.3	9.8	0.5	4.9	8.9	NC	0.7	0.4	0.6
Urethra	32.0	NS	NS	5.7	NC	NC	0.8	NC	NC
Uterus, whole	31.9	8.4	0.6	5.7	7.7	NC	0.8	0.4	0.7
Endometrium	30.6	13.4	0.8	5.5	12.2	NC	0.8	0.6	1.0
Vagina	20.8	8.3	NS	3.7	7.6	NC	0.5	0.4	NC

BLQ=below limit of accurate quantification (<0.062 µg equivalents/g); NA=not applicable; NC=not calculable (tissue is BLQ/NS at this time); NS=no sample, tissue not sectioned.
*Data are µg equivalents of lefamulin per gram tissue

Figure 2. Distribution of Radioactivity in Female Rats Following IV Administration of [14C]-Lefamulin



IV=intravenous; H&E=Haemotoxylin and Eosin; QWBA=quantitative whole-body autoradiography.
*Under bright field conditions, radioactive areas (silver grains) show as small particles and the tissue histology can be viewed. Under dark field conditions, silver grains show up bright white against a dark, false-color background.

CONCLUSIONS

- [^{14}C]-lefamulin distributed rapidly and in a homogenous manner down to the cellular level into relevant STI urogenital tract tissues of rats with high tissue/blood ratios
- Lefamulin exhibited rapid distribution into various urogenital tract tissues comparable to that observed into lungs
- Based on the tissue distribution and potent *in vitro* activity of lefamulin against the most common bacterial causes of STIs, including multidrug resistant strains, further assessment of lefamulin for the treatment of STIs is warranted

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Disclosures

Wolfgang W. Wicha and Steven P. Gelone are employees of Nabriva Therapeutics. Claire Henson and Kate Webbley are employees of Pharmaron UK Ltd, who were contracted by Nabriva to perform these studies.

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