Inhaled Liposomal Ciprofloxacin for the Prevention and Treatment of Severe Intracellular Lung Infections

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ABSTRACT

Aradigm has developed liposomal ciprofloxacin, Lipoquin® (LIP), and Pulmaquin® (PUL), a mixture of LIP and free ciprofloxacin (FCI), to treat lung infections. PUL and LIP have significant activity against the extracellular lung infections with *Pseudomonas aeruginosa* in CF and non-CF bronchiectasis patients.

Intracellular infections that persist after uptake by macrophages are more difficult to treat; certain types of liposomes are avidly taken up by pulmonary macrophages. We therefore tested PUL & LIP for their efficacy in difficult intracellular infections.

PUL and LIP are efficacious in mouse models of post-exposure prophylaxis for severe lung infections with potential bioterrorism agents: tularemia, pneumonic plague, and Q fever. E.g., a single dose of aerosolized LIP administered 24-hr post challenge provided 100% protection against virulent *Francisella tularensis* strain Schu S4.

LIP and PUL are also efficacious in mouse models of *Mycobacterium avium subsp hominissuis* (MAH) and *M. abscessus* (*M ab*) lung infections over 3 and 6 weeks (w). A once-daily 1 mg/kg lung dose of ciprofloxacin had significant ($p<0.05$) reductions in colony forming units (CFU) in the lung. For MAH, LIP reduced CFU by 49% (3w) and 78% (6w); PUL reduced CFU by 45% (3w) and 70% (6w). For *M ab*, LIP reduced CFU by 99.0% (2 log, 3w) and 99.95% (>3log, 6w); PUL reduced CFU by 99.2% (>2 log, 3w) and 99.88% (~3log, 6w). There was no evidence of emergence of bacterial resistance or adverse findings.

Thus, PUL and LIP are promising therapies for severe intracellular lung infections.

OBJECTIVES

To treat lung infections, Aradigm has developed two liposomal ciprofloxacin formulations:

- Lipoquin (LIP) (liposomal ciprofloxacin alone)
- Pulmaquin (PUL), a mixture of Lipoquin and free ciprofloxacin (FCI).

Pulmaquin and Lipoquin have significant activity against the extracellular lung infections with *Pseudomonas aeruginosa* in CF and non-CF bronchiectasis patients; PUL has completed Phase 3 clinical trials in non-CF bronchiectasis patients infected with *P aeruginosa*.

The data in this poster support the efficacy of LIP and PUL at a clinically relevant dose of 1 mg/kg against intracellular lung infections that persist after uptake by macrophages and are more difficult to treat; certain types of liposomes are avidly taken up by pulmonary macrophages. These infections are:

- Potential bioterrorism agents: *Francisella tularensis* (inhalational tularemia) and *Yersinia pestis* (pneumonic plague).
- Non-TB mycobacteria (NTM: *Mycobacterium avium subsp hominissuis* (MAH) and *M. abscessus.*

METHODS/RESULTS

Efficacy against highly virulent *F. tularensis* Schu S4 for inhalational tularemia (1):

Female BALB/c mice (n=12/group) were lethally challenged with *F. tularensis* Schu S4 via the aerosol route (~10 CFU/animal)

24 hours post challenge received a single treatment:

- 1 mg/kg lung dose of inhaled Lipoquin aerosol: LIP (IH) (dose expressed as HCl salt)
- 50 mg/kg of intranasal Lipoquin: LIP (IN)
- 50 mg/kg of oral ciprofloxacin: CIP (Oral)
- Intranasal PBS: PBS (IN)

Mice were monitored for 20 days post-challenge

Fig 1. LIP (IN) had only 1/12 survival, but significantly ($p<0.005$) increased time to death of infected mice vs. control [PBS (IN) or CIP (Oral)].

LIP (IH) had 100% survival and significantly ($p<0.005$) increased survival vs. control [PBS (IN)), CIP (Oral)], or LIP (IN).

Efficacy against *Y. pestis* CO92 for pneumonic plague (2):

Female BALB/c mice (n=10/group for survival and n=5/group for bacterial burden) were challenged via the aerosol route with approximately $1x10^4$ CFU *Y. pestis* strain CO92 and treated 24 hours post-challenge with:

- 1 mg/kg lung dose inhaled LIP or PUL (IH) (dose expressed as HCl salt)
- Inhaled empty liposome control: ELC (IH)
- 30 mg/kg intraperitoneal ciprofloxacin: CIP (IP)
- 300 µl PBS (IP)

All treatments improved survival compared to PBS (IP) or ELC (IH) ($P<0.001$) (**Fig. 2**).

Fig 2A: Survival for 1 dose: LIP IH 100% ($p<0.029$ vs. CIP IP); PUL IH: 80%; CIP IP: 60%.

Fig 2B: Survival for 3 days of therapy all treatments 100%

Fig. 3: Spleen: 1 dose of all antibiotics significantly ($p=0.023$) reduced bacterial load (colony forming units, CFU) < limit of detection (LOD) and vs. controls PBS IP or ELC IH

For lung: 1 dose of LIP and PUL significantly ($p<0.023$) reduced CFU < LOD and vs. ELC.

1 dose of CIP IP significantly ($p=0.023$) reduced lung CFU vs. PBS IP, but bacteria found in 2/5.

There were no significant differences in lung CFU among the different therapies.

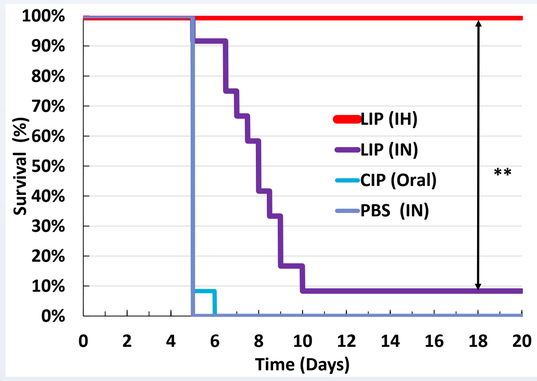
Efficacy against *Mycobacterium avium subsp hominissuis* (MAH) and *M. abscessus* (3 & 4):

- For MAH, C57BL/6 mice (n=10/group/time point) were infected by intranasal instillation (IN) with 5 x 10^8 MAC 104 strain of MAH.
- For *M abscessus*, C57 beige bg/bg mice (n=12/group/time point) were infected by IN with 5.4 ± 0.3 x 10^7 *M. abscessus* strain 101.
- One week later (Week 0), therapy was initiated with LIP, PUL, or FCI at a ciprofloxacin lung dose of 1 mg/kg (dose expressed as HCl salt) and delivered daily IN for 3 and 6 weeks.
- Controls were saline and ELC with the lipid dose matching the lipid content of the 1 mg/kg LIP dose.
- At the end dosing, bacterial burden (CFU) was measured in lungs and spleens.

Fig 4: LIP and PUL significantly reduced lung CFU at 3w vs. saline and at 6w vs. CFU at 3w and saline. For MAH, LIP reduced CFU by 49% (3w) and 78% (6w); PUL reduced CFU by 45% (3w) and 70% (6w). For *M abscessus,* LIP reduced CFU by 99.0% (2 log, 3w) and 99.95% (>3 log, 6w); PUL reduced CFU by 99.2 (>2 log, 3w) and 99.88% (~3 log, 6w).

There was no evidence of emergence of bacterial resistance or adverse findings.

Fig 1: Survival of mice challenged with *F. tularensis* Schu S4 and treated with a single dose of antibiotic†



** p< 0.005 for increased survival vs. other groups

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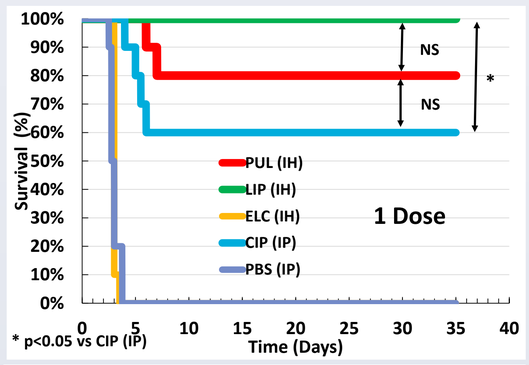
Fig. 2: Survival of mice challenged with *Y. pestis* CO92 following A, 1 dose and B, 3 days of therapy†



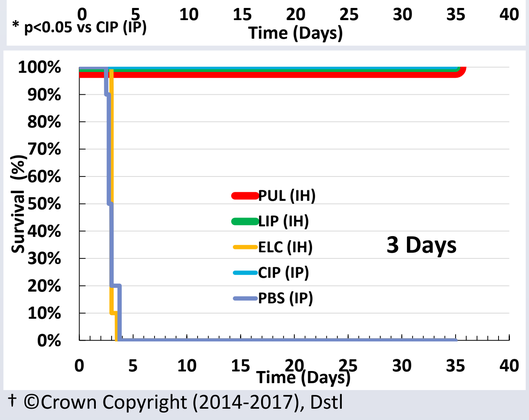


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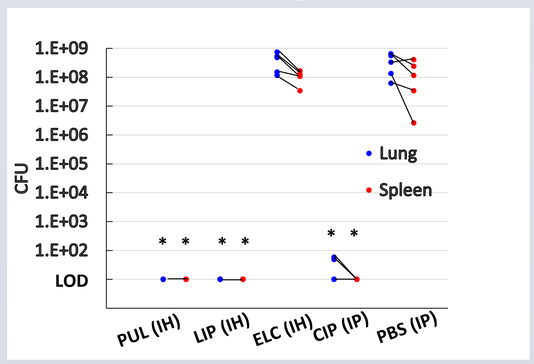
Fig 3: *Y. pestis* CO92 bacterial burden in lung & spleen after single dose of therapy at onset of clinical signs in control groups (~2.5 days post-challenge)†



* p<0.05 vs ELC for LIP or PUL or PBS IP for CIP IP

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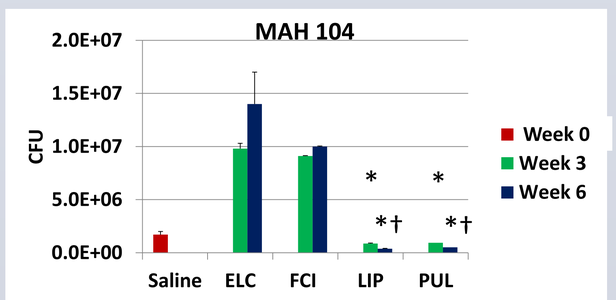
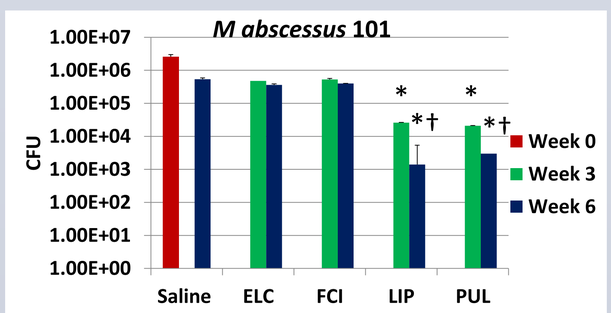
Fig. 4. Lung Bacterial Burden for MAH and *M. abscessus:*





Values are mean ± SD
CFU: colony forming units; ELC: Empty Liposome Control; FCI: Free Ciprofloxacin for Inhalation;
*** p<0.05 compared to CFU of saline control at Week 0**
† p<0.05 compared to CFU at Week 3

CONCLUSIONS

Both liposome ciprofloxacin formulations (LIP and PUL) are efficacious.

- For *F tularensis*, a single dose of LIP increased survival vs. oral ciprofloxacin
- For *Y pestis,* a single dose of LIP or PUL increased survival and reduced bacterial load in lungs and spleen
- For MAH and *M abscessus:*
  - Both LIP and PUL significantly reduced bacterial load in lungs, whereas ciprofloxacin alone was ineffective.
  - For both LIP and PUL, the decrease in lung CFU at Week 6 was significantly greater than at Week 3; thus, there is progressive improvement with time. Since the treatment in humans is typically many months, this finding is encouraging.
  - No emergence of resistance or adverse findings were observed.

Thus, both PUL and LIP are promising therapies for severe intracellular lung infections.

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