Metaphylaxis vs. curative treatment for antibiotics: why we need different dosage regimen

Pierre-Louis Toutain,
Ecole Nationale Vétérinaire
INRA & National veterinary School of Toulouse, France
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Terms to describe herd or flock antibiotic use

- Disease
- Health

**Therapy**
- Administration of an AB to an animal, or group of animals, which exhibit clinical disease

**Metaphylaxis** (Control)
- Administration of an AB to animals, usually as a herd or flock, in which morbidity and/or mortality has exceeded baseline norms.
  - Hazard present

**Prophylaxis** (prévention)
- Administration of an AB to exposed healthy animals considered to be at risk, (before onset of disease)
  - Risk factor present

**Growth promotion**
- Administration of an antimicrobial, usually as a feed additive, to growing animals that results in improved physiological performance.
Terminology and risk communication

- Metaphylaxis
  - Mass medication
    - Very negative
  - Very early treatment
    - Should be positive
2-Why metaphylaxis
The pro

• **Convenience**
  – Possible administration by the oral route to a group of animals (pen, herd…) i.e. collective treatments

• **Medical reasons**
  – No alteration of physiological function
  – No or minimal depression of natural mechanisms of defense
  – Prevent possible alteration of the disposition of the AMD
  – Better cure rate

• **Animal welfare**
  – All animals determined to be at an unacceptable high risk of developing a bacterial disease
  – No subsequent lesion (lungs…)

• **Economical reasons**
  – Increase the average daily gain (ADG)
Fever: water vs feed intake

Challenge with Actinobacillus Pleuropneumonia Toxins

Influence of disease on PK of orally administered OTC (50 mg/kg)

Before challenge

After challenge
The cons

• Public health issues
• Overuse of antimicrobial drugs favouring the selection of resistant bacteria
  – Actually never demonstrated
  – Not a relevant endpoint that is the impact on gut microbiota
The most relevant endpoint is not the AMD consumption but the collective impact on commensal microbiota

- Further studies should now investigate, at group level, the impact on the overall consumption of antibiotic vs. the impact (the selection of resistance) on the gut microbiota (both treated and not treated)
Metaphylaxis: the point of view of the microbiologist
The point of view of the microbiologist

Disease  health

Antibiotic consumption

Therapy  Metaphylaxis (Control)  Prophylaxis (prévention)  Growth promotion

Pathogen load

High  Small  No  NA
Pathogen load: Wild and mutant subpopulations
Our Hypothesis on the influence of FQ on the emergence of resistance in the target flora

Hypothesis: metaphylaxis is more desirable in terms of emergence of resistance than a conventional curative treatment.
Our set of working hypothesis

- Efficacious dosage regimen is different when the pathogen load is large, low or null: the so-called inoculum effect
- The likelihood of resistance is less with metaphylaxis than with those associated to therapeutic treatment
- The appropriate dose should be different
The inoculum effect: in vitro evidences
MICs estimated with different inoculum densities, relative to that MIC at $2 \times 10^5$

- Ciprofloxacin
- Gentamicin
- Linezolid
- Daptomycin
- Oxacillin
- Vancomycin

Figure 3. MICs estimated with different inoculum densities, relative to that MIC at $2 \times 10^5$. These estimates were obtained from cfu data; when the viable cell density at 18 h was approximately equal to that in the initial inoculum.

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Functional relationship between bacterial cell density and the efficacy of antibiotics
Inoculum at $10^6$ cfu/mL
- 99.9% killing after 2 h, at the concentration of $16 \times$ MIC.

Inoculum at $10^8$ cfu/mL
- No bactericidal activity at 2–16 $\times$ MIC

Similar results with different penems
• The case of marbofloxacine
In vitro dynamic system

- Thermostat: 37°C
- Pump
- Sampling
- Fresh broth
- Broth + bacteria + antibiotic
- Magnetic stirrer
- Water bath
- Waste
In vitro dynamic system

- Marbofloxacin concentrations profiles in an in vitro dynamic system
Marbofloxacin and the selection window

Interaction *in vitro* between $T_{MSW}$ and inoculum size

• Selection of resistant bacteria when:
  – When marbofloxacin concentrations are within the mutant selection window
  – With a higher frequency in higher bacterial inoculum size
The inoculum effect: in vivo investigations
Hypothesis:
- the bacterial load at the infection site impact the PK/PD parameters (AUC/MIC) of fluoroquinolones (marbofloxacin).

Methods
- rat lung infection model, *Klebsiella pneumoniae*.
- we measured the influence of different marbofloxacin dosage regimens on selection of resistant bacteria
  - low (10^5 CFU) vs. a high (10^9 CFU) inoculum size

Results: prevention of resistance
- (AUC)/MIC ratio of 189 h for the low inoculum
- AUC/MIC ratios up to 756 h for the high inoculum.
Rodent model of metaphylaxis
Impact of early versus later fluoroquinolone treatment on the clinical; microbiological and resistance outcomes in a mouse-lung model of Pasteurella multocida infection

Aude A. Ferran, Pierre-Louis Toutain, Alain Bousquet-Mélou*

UMR181 Physiopathologie et Toxicologie Expérimentales, INRA, ENVT, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, BP 87 614, 31076 Toulouse Cedex 3, France
Objectives:

- To assess the impact of early (metaphylaxis, control) versus later fluoroquinolone treatment on:
  - The **clinical cure** (survival of mice)
  - The **microbiological cure** (bacterial eradication)
  - the **resistance outcomes** (selection of resistant (target) bacteria)
Materials and methods

Model of pulmonary infection

Inoculation of *Pasteurella multocida*
1500 CFU/lung

A strain of *Pasteurella multocida* isolated from the trachea of a pig with clinical symptoms of a bacterial lung infection
Model of pulmonary infection

Inoculation of Pasteurella multocida 1500 CFU/lung

Progression of infection

Bacteria counts per lung (CFU/lung)

18 control mice were used to assess the natural growth of Pasteurella multocida in the lungs.
Time of marbofloxacin administration

Progression of infection

Inoculation of Pasteurella multocida 1500 CFU/lung

Bacteria counts per lung (CFU/lung)

- no clinical signs of infection
- anorexia
- lethargy
- dehydration

early administration

late administration
Marbofloxacin: Doses administered

Inoculation of *Pasteurella multocida* 1500 CFU/lung

10 hours after the infection (n=14)
- A single administration of marbofloxacin
- Two doses tested for each group: 1 mg/kg and 40 mg/kg

32 hours after the infection (n=14)
Pharmacokinetic study

Inoculation of Pasteurella multocida 1500 CFU/lung

One administration of marbofloxacin (20 mg/kg)

10 hours after the infection

32 hours after the infection
Results

Marbofloxacin IP administration at 20mg/kg

Exposure was 3-times higher for the late group than for the early treated group.

Late treatment
Clearance=6.2 ml/kg/min

Early treatment
Clearance=20.8 ml/kg/min
Endpoints measured

Inoculation of *Pasteurella multocida* 1500 CFU/lung

10 hours

48 hours after inoculation

Counting of bacteria 38 hours after marbofloxacin administration

32 hours

70 hours after inoculation
1-Clinical outcome (survival)

Marbofloxacin administrations

% mice alive

control

1 mg/kg

40 mg/kg

Marbofloxacin doses
2-Bacterial eradication only the early high dose

Marbofloxacin administrations

Early  Late  Early  Late

% of mice with bacterial eradication

control  1 mg/kg  40 mg/kg

Marbofloxacin doses
2-Bacterial eradication

Early low dose = late high dose

Marbofloxacin administrations

Early

Late

% of mice with bacterial eradication

1 mg/kg

40 mg/kg

Marbofloxacin doses
3-Selection of resistant (target) bacteria

Marbofloxacin administrations

High mortality in this group & impossibility to see if resistant bacteria

% of mice with resistant bacteria

Early  Late  Early  Late

control  1 mg/kg  40 mg/kg

Marbofloxacin doses

☆ No resistant bacteria
Conclusions

1. In the present study, the early administration of 1 mg/kg marbofloxacin gave a higher survival rate and a similar percentage of bacterial eradication as the late administration of 40 mg/kg marbofloxacin.

2. If considering emergence of resistance, the likely optimal regimen should be an early treatment (slightly) higher than 1 mg/kg
Low or High Doses of Cefquinome Targeting Low or High Bacterial Inocula Cure Klebsiella pneumoniae Lung Infections but Differentially Impact the Levels of Antibiotic Resistance in Fecal Flora

Maleck V. Vasseur, Michel Laurentie, Jean-Guy Rolland, Agnès Perrin-Guyomard, Jérôme Henri, Aude A. Ferran, Pierre-Louis Toutain and Alain Bousquet-Mélo

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Metaphylaxis vs. curative

• Pulmonary infectious model by inhalation (*P. multocida*)
• Amoxicillin & et cefquinome
• Treatment during the prepatent (incubation) period (24h) vs. when symptoms are present
Effect of amoxicillin (clinical cure) metaphylaxis vs. curative

Early/metaphylaxis

Late/curative

Dose mg/kg

2.5  5  25  50

80   100   51  100
Effect of amoxicillin (bacteriological cure) metaphylaxis vs. curative

![Graph showing the effect of different doses of amoxicillin on early and late bacterial cure.](graph.png)
Effect of cefquinome (clinical cure) metaphylaxis vs. curative
Effect of cefquinomine (bacteriological cure) metaphylaxis vs. curative
Impact on gut microbiota
Why gut microbiota

• Any antibiotic treatment can impact the gut microbiota (commensal flora)
• Gut microbiota is the main location for the genesis of resistant bacteria and it constitute the main pool of genes of resistance
• It is a public health objective to mitigate the impact of any antibiotic on the gut microbiota
• An optimal dose regarding the target pathogen can be detrimental to the gut microbiota
One world, one health

Commensal flora
Genes of resistance (zoonotic pathogens)

Environment

Food chain

Commensal flora

Greening our AB
Example of conflict of interest

- the optimal dose in terms of pathogen eradication was detrimental to the gut microbiota
Selectivity of antimicrobial drugs in veterinary medicine

Selectivity

PD: Rather Low potency
    Narrow spectrum

PK: Selective distribution of the AB to its biophase
Impact of antibiotics on the gut microbiota is dose-dependent

Correlation between Fecal Concentrations of Ciprofloxacin and Fecal Counts of Resistant Enterobacteriaceae in Piglets Treated with Ciprofloxacin: toward New Means To Control the Spread of Resistance?

Thu Thuy Nguyen, Elisabeth Chachaty, Clarisse Huy, Carole Cambier, Jean de Gunzburg, France Mentré, and Antoine Andremont
Impact of antibiotics on the gut microbiota is dose-dependent

Ciprofloxacin: 1.5 or 15mg/kg/days
In vitro assessment of the selectivity of antibiotics on the target pathogen vs. commensal flora: eradication of a low vs. high inoculum size of \textit{P multocida}
Amoxicillin has a good selectivity regarding E coli when eradicating a low but not a high inoculum size of lung *P. multocida*

- **Low:** $10^5$ CFU/mL
- **High:** $10^7$ CFU/mL

**SI** = 51

**P. Multocida** ($10^5$ or $10^7$ CFU/ml)

**E coli** ($10^7$ CFU/mL)
Cefquininome has no selectivity regarding E. coli when eradicating either a low or a high inoculum size of lung *P. multocida*

Low: $10^5$ CFU/mL

High: $10^7$ CFU/mL

**SI** = 2.9

**SI** = 0.66

*P. Multocida* ($10^5$ or $10^7$ CFU/ml)

*E coli* ($10^7$ CFU/mL)
Impact of early versus later fluoroquinolone treatment on the clinical and microbiological outcomes in calves challenged with *Mannheimia haemolytica*
Experimental challenge with *M. haemolytica*

- **Calves**
  - $N=32$;
- **Bacteria strain**
  - *M. haemolytica* (MIC 0.03 µg/mL)
- **Challenge**
  - Intratracheal injection,
  - $10^7$ CFU tot/calf
- **Inclusion criteria**
  - Rectal $T^\circ C$ recording every 3h after inoculation
  - increase temperature $>1^\circ C$ of basal individual temperature mean (before challenge)
Experimentation

Control

E2 (Early, 2mg/kg) group

L2 (Late, 2mg/kg) group

L10 (Late, 10mg/kg) group

No treatment

Marbofloxacin 2mg/kg, 2-4h post-inclusion

Marbofloxacin 2mg/kg (L2) or 10mg/kg (L10) 36-38h post-inclusion

Evolution of the bacterial load in the lower respiratory tract
PCR in lung tissues samples
(110h after an experimental lung infection *P. haemolytica*)

**Early +12h**
**Late +24-36h**

![Graph showing ADN copy variation with different treatments and time points.]

- **Control:** 2mg/kg
- **E2:** 2mg/kg
- **L2:** 10mg/kg
- **L10:** 10mg/kg
Detection of *M. haemolytica* in BAL

![Graph showing the detection of *M. haemolytica* in BAL over time with different treatments. The graph plots the number of copies (log 10/mL) against time (h). The treatments include Control, E2, L2, and L10.](image-url)
Pulmonary lesions

- Typical lesions of *M. haemolytica*
- Moderate extension and severity
- Increased frequencies in control and L2 groups
Discussion

- **E2 vs L2**
  - Early treatment $\Rightarrow$ fast eradication of bacterial load
  - No pulmonary lesions

- **E2 vs L10**
  - Equivalence on bacteriological and clinical issues $\Rightarrow$ how perform a fast assessment of the bacterial charge to adjust antibiotic regimen ??

- **Sustainability**
  - Repeatability among pathogens and molecules?
  - Evaluating the treatment « window »
  - Impact of the lower dose on commensal flora
Difficulties for a very early treatment with a lower dose

- Early diagnostic
- Regulatory considerations
- Marketing consideration
Fever Alert:
fever tags, intraruminal transponders, eye temperature, locomotor activity...
Regulatory considerations

- Difficulty to manage two dosage regimen
- EMA is against the principle
- Difficulty to establish a dosage regimen using a dose titration
Conclusions (1) efficacy

• For 3 antibiotics (marbofloxacin, cefquinome and amoxicillin), it was shown that the efficacious dose (clinical and bacteriological endpoints) was lower when treatment is initiated early
Conclusion (2)

Resistance selection

• In the case of resistance selection at the infectious site, for a given dose, early treatments were always associated with less selection for resistant bacteria than the late treatments.
Conclusions (3)
gut flora

- Using a lower doses thanks to an early treatment can improve the selectivity of antibiotics regarding the gut flora
Conclusion (4)  
other expected effects of a low dose

– Reduction of the overall antibiotic consumption
– Limitation of the environmental contamination
Overall conclusion on metaphylaxis

• It is not acceptable to condemn metaphylaxis (control) by the argument that it is a collective treatment i.e. ineluctably as an overuse of antibiotics especially if we are in position to optimise (decrease) dosage regimen and condition of use of this kind of administration.