The Oral Administration of Antibiotics to Research Mice

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Antibiotic administration to mice in biomedical research:

1. **Antibiotic use is part of standard veterinary care**

2. **Use of immunosuppressed animals increases the risk of bacterial infection**

Administration of antibiotics (and other meds) in the drinking water is desirable for several reasons, including :

Low stress to the animal

Efficient in time for researchers and clinical staff

Medication administration in the drinking water of mice

Potential limitations:

1. **Drug stability in the water**
2. **Mouse consumption of water**
3. **Systemic absorption**

Previous studies:

McIntyre and Lipman (2007) tested Clavamox and TMS stability over 7 days:

	Reverse osmosis water	Acidified
Amoxicillin	Stable	↓then stable
Clavulanic Acid	Gradual decrease	Immediately gone
Sulfamethoxazole Trimethoprim	Precipitation caused variation between timepoints	

Measured serum antibiotic levels after food delivery and had very low serum concentrations

Purpose:

Specific Aim 1

Test the stability of enrofloxacin and doxycycline in the drinking water of mice (tap and acidified water)

Specific Aim 2

Measure consumption of medicated water for these antibiotics, plus amoxicillin and TMS

Measure plasma antibiotic levels and compare with MIC values of murine pathogens

Antibiotic	Dosage	mg/mL of drinking water	Source
Enrofloxacin	50 mg/kg/day	0.25 mg/mL	Injectable
Doxycycline	10 mg/kg/day	0.05 mg/mL	Oral suspension
Amoxicillin	50 mg/kg/day	0.25 mg/mL	Oral suspension
TMS	160 mg/kg/ml	0.8 mg/mL	Oral suspension

Dosing based on mice drinking 5.0 mL of water per day

Doses of Enrofloxacin, Amoxicillin and TMS are high to account for allometric scaling

Specific Aim 1: The stability was tested in tap and acidified water (pH 2.5-3.0) over the course of a week, in water bottles suspended in empty mouse cages. Water was collected to mimic the water the mice would be drinking from the end of the sipper tube.

High-performance liquid chromatography(HPLC) analysis:

- **Day 0- four 10-mL samples**
- **Day 7-four additional 10-mL samples**
- **Shimadzu LC with diode array detector**

Results:

Enrofloxacin

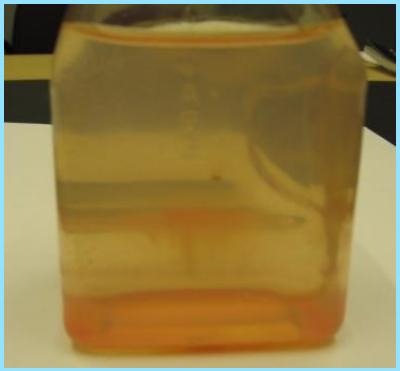
Values in % recovery

Water type	Day 0	Day 7
Tap	95.6 ± 0.5%	94.9 ± 5.2%
Acidified	100.3 ± 3.0%	99.2 ± 3.8%

Oral Doxycycline (Vibramycin)

Values in % recovery

Water type	Day 0	Day 7
Acidified	97.2 ± 4.5%	87.3 ± 5.2 %
Tap	34.6 ± 4.1%	35.1 ±13.1%
	Precipitate present	



Tap water acidified 90.8 ± 0.1% Recovery

Doxycycline Powder Chemical Grade

Values in % recovery

Water type	Day 0	Day 7
Acidified	74.6 ± 1.0%	84.4 ± 10.2%
Tap	104.4 ± 4.5%	64.3 ± 6.5%

Discussion points: Specific Aim 1

1. **Enrofloxacin is stable for 7 days in both tap and acidified water.**

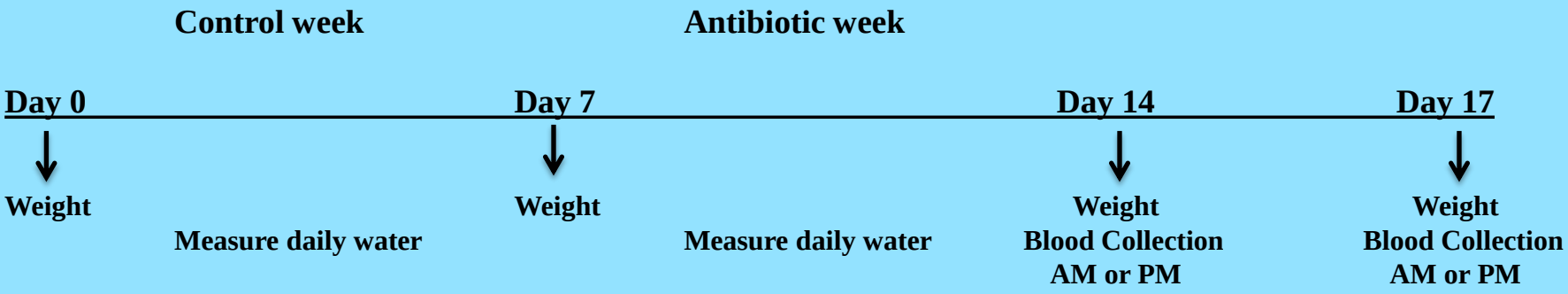
2. **Oral doxycycline (Vibramycin) precipitates out in pH 7.0 water, leaving much unavailable to the mouse.**

3. **Chemical grade has much greater bioavailability, but needs to be declared as chemical grade in IACUC protocols.**

Specific Aim 2

Test water consumption and plasma antibiotic concentrations achieved

Female C57Bl/6 mice
4 groups (n=8)



Plasma antibiotic concentrations measured by HPLC

Results: Water consumption

Antibiotic	Control water consumption (ml per two mice per day)	Antibiotic water consumption (ml per two mice per day)
Enrofloxacin	9.7±0.8	11.4±0.3*
Doxycycline	9.3±0.3	10.1±0.9
Amoxicillin	9.3±0.4	8.8±0.8
Trimethoprim	10.1±1.3	11.2±1.5

* Only Enrofloxacin consumption changed significantly between the two weeks

The only significant difference in body weights were in the TMS group

Results: Plasma antibiotic concentrations

Antibiotic	AM plasma antibiotic concentration (ng/ml of plasma)	PM Plasma antibiotic concentration (ng/ml of plasma)
Enrofloxacin	112.2±11.7	140.1±10.4
Doxycycline	56.6±12.5	42.9±7.8
Amoxicillin	299.2±64.1	275.2±50.2
Trimethoprim	5.7±2.3	5.9±1.2

Results: Plasma antibiotic concentrations

Note the units: ng/ml

Minimum Inhibitory Concentration for most bacteria/antibiotic concentrations

µg/ml

The plasma concentrations of the antibiotics are approximately 10% of desired values

Results: Plasma antibiotic concentrations

Results consistent with the McIntyre and Lipman paper with antibiotics delivered in the food.

Additional study: Enrofloxacin

1 AM blood collection

Double enrofloxacin dose with 1 AM collection

Results: Plasma concentrations

Antibiotic	1 AM plasma antibiotic concentration (ng/ml of plasma)	Double dose 1 AM Plasma antibiotic concentration (ng/ml of plasma)
Enrofloxacin	117.5±16.9	174.8±55.5

Discussion points: Specific Aim 2

Administration of antibiotics in the drinking water of mice does not achieve adequate plasma concentrations to be reliably effective against most pathogenic bacteria.

Bolus dosing, either parentrally or entrally is more likely to achieve therapeutic concentrations, especially for concentration dependent antibiotics.

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Thank you for your time.

Questions?