EPAA 3Rs Awards and Grants
2021
REFINEMENT PRIZE
2021

HaPILLnes
PRECISE VOLUNTARY ORAL DRUG DOSING IN RODENTS - AN INNOVATIVE 3Rs APPROACH
Laboratory Animal Sciences

The science and technology dealing with the procurement, breeding, care, health, and selection of animals used in biomedical research and testing.

3R’s
- REPLACE: Replace animal studies with other methods
- REDUCE: As many trials as required, as few as possible
- REFINE: Minimize stress of study animals

Refinement aims to minimize animal harm while maximizing scientific validity.

Diagram:
- Benefit potential
- Harm potential
- 3R’s

Replace animal studies with other methods
As many trials as required, as few as possible
Minimize stress of study animals

Refinement aims to minimize animal harm while maximizing scientific validity.
The observed poor pre-clinical translation and reproducibility means we, researchers, need to reinforce the implementation of 3R’s principles. Why?
Because ≈ 9 million animals are used in E.U. every year for research purposes

Adapted from: Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017
In humans

Oral dosing is part of the daily routine for millions of laboratory animals

In pre-clinical studies
Nowadays:

GAVAGE

1. Restrain the animal
2. Insert cannula into the animal’s stomach
3. Press the syringe plunger to inject the drug to the animal’s stomach

Gavage induces physiologic stress.
Thus, gavage conditions the observed metrics in pre-clinical studies.
Over the years, many alternatives have been developed such as:

- Drug suspension in sucrose solution and voluntary syringe feeding
- Fast dissolving oral drug biofilm and voluntary syringe feeding
- Drug given in highly caloric and palatable substances (e.g., Nutella, peanut butter, sugar paste)
- Drug given in food/drinking water

**Limitations**

- Lack of dose precision
- Requires single-housing
- Requires animal restraint
- Metabolically disruptive
So, we proposed ourselves to find a truly voluntary, stress free and metabolic inert alternative

To help implement the 3R’s principles of Refinement and Reduction in oral drug dosing
1. PILL TECH VOLUNTARY CONSUMPTION
2. PRECISE ORAL DOSING
3. STRESS FREE
4. METABOLIC INOCUITY
5. SAFETY
PILL TECH VOLUNTARY CONSUMPTION
C57BL/6 mice

Wistar rats

C57BL/6 mice (n= 6 experimental group). Data are presented as mean ± s.e.m.
C57BL/6 mice (n= 6 experimental group). Data are presented as mean ± s.e.m.
PILL TECH VOLUNTARY CONSUMPTION

Animal model of disease:
cuprizone intoxication multiple sclerosis model

- Control + empty-PILL
- MS mouse model + empty-PILL
- MS mouse model + Sitagliptin PILL-dosed (50mg/Kg )
PRECISE ORAL DOSING

CS7BL/6 mice (n=5 experimental group). ANOVA one way. **** p<0.0001 vs control.
Data are presented as mean ± s.e.m.
STRESS FREE

Open Field Test

- Control
- PILL Formula A

Elevated Plus Maze

- Control
- PILL Formula A

Data are presented as mean ± s.e.m.

C57BL/6 mice (n= 6 experimental group).
STRESS FREE

Y-Maze test
- Control
- PILL Formula A

Splash test
- Control
- PILL Formula A

Direct physiologic readout

Plasmatic Corticosterone (ng/mL)
- Control
- Gavage
- PILL Formula A

Acute: 30min postgavage

C57BL/6 mice (n=6 experimental group). Data are presented as mean ± s.e.m.
METABOLIC INOCUITY

- **Glucose (mg/dL)**
  - Control
  - PILL Formula A

- **Triglycerides (mg/dL)**
  - Control
  - PILL Formula A

- **Total cholesterol (mg/dL)**
  - Control
  - PILL Formula A

- **HDL cholesterol (mg/dL)**
  - Control
  - PILL Formula A

- **LDL cholesterol (mg/dL)**
  - Control
  - PILL Formula A

- **Body weight (g)**

- **Emáx (mN)**

*CS7BL/6 mice (n=6 experimental group). Data are presented as mean ± s.e.m.*
SAFETY

Liver function profile

- ALT (mg/dL)
- AST (mg/dL)
- Albumin (mg/dL)

Kidney function profile

- Creatinine (mg/dL)
- Uric acid (mg/dL)
- Serum Urea (mg/dL)

C57BL/6 mice (n=6 experimental group). Data are presented as mean ± s.e.m.
SAFETY

Histology of liver and kidney sections

Control

Vehicle

Liver

Kidney

100 µm
PATENT COOPERATION TREATY

Sara Nunes, PhD student
André Alves, PhD student
Pedro Vieira, MSc
Carolina Ferreira, MSc
Beatriz Ormonde, MSc student

Sofia Viana, PhD Inventor/supervisor
Inês Preguiça, HaPILLness PhD student
Flávio Reis, PhD Inventor/Cosupervisor

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