



A quick and low-intensity method for oral administration to large numbers of mice: A possible alternative to oral gavage

Laboratory Animals
0(0) 1–6
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/00236772211035250
journals.sagepub.com/home/lan



Tiago Neto^{1,2,3} , Ana I Faustino-Rocha^{3,4,5} ,
Rui M Gil da Costa^{2,3,6}, Rui Medeiros^{2,7,8,9,10} and
Paula A Oliveira^{3,11}

Abstract

Oral administration of medication to experimental animals is a cause of significant stress. When coupled to animals who are already under strenuous circumstances due to the disease being modelled, there is a significant risk for increased morbidity and mortality, thus influencing the results. Faced with these constraints, a low-intensity method for oral administration was developed, based solely on the natural behaviour of the animals and minimal conditioning, in which precise doses of medication were administered in a locally available, standard wheat cookie fragment, providing both a palatable vehicle and an absorbent matrix for the medication. Fast administration to large numbers of animals was thus achieved, safeguarding the animals' welfare and ensuring ease of handling. This method is a promising alternative to oral gavage in pre-clinical drug studies with laboratory mice.

Keywords

Cookie, fast, mouse, stress, oral administration, oral gavage, welfare

Date received: 11 April 2021; accepted: 6 July 2021

Introduction

Mice models have emerged as the tool of choice for basic research.¹ They are easily stressed, and stress *per se* may modify food intake, energy expenditure, physical activity, and body composition.^{2–5} The degree of stress experienced by the animals may be influenced by the number of researchers involved in the animals' manipulation, as well as their skill and experience working with mice.⁶

When housed under controlled conditions of light–dark cycle (12h light/12h dark), mice consume most of their food (almost 70%) during the dark period, with short feeding bouts during the light period. Water consumption is linked to food intake and declines in fasted or food-restricted animals. Additionally to the light–dark cycle, mice feeding behaviour may be also influenced by other variables, like room temperature, humidity, noise, biological differences, and social stratification.⁷

Oral administration of investigational medicinal products to mice is usually performed. Some

¹Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal

²Molecular Oncology and Viral Pathology Group, CI-IPOP, Portuguese Institute of Oncology (IPO), Portugal

³Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Portugal

⁴Department of Zootechnics, School of Sciences and Technology, University of Évora, Portugal

⁵Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal

⁶Postgraduate Programme in Adult Health (PPGSAD) and Tumour Biobank, Federal University of Maranhão (UFMA), Brazil

⁷Faculty of Medicine, University of Porto, Portugal

⁸Virology Service, Portuguese Oncology Institute of Porto (IPO Porto), Portugal

⁹Biomedical Research Center (CEBIMED), Faculty of Health Sciences of the Fernando Pessoa University, Portugal

¹⁰Research Department, Portuguese League Against Cancer - Regional Nucleus of the North (Liga Portuguesa Contra o Cancro - Núcleo Regional do Norte), Portugal

¹¹Department of Veterinary Sciences, UTAD, Portugal

Corresponding author:

Ana Faustino, University of Trás-os-Montes and Alto Douro Quinta de Prados Vila Real, 5001-801 Portugal.

Email: anafaustino.faustino@sapo.pt

compounds may affect the odour or flavour of food or water, causing animals to avoid consuming them and changing the amount of the test compound ingested by each animal.⁸ Oral gavage is a widely used alternative method for administering test compounds to animals by introducing them in liquid form directly into animals' stomachs using a special feeding needle.^{9–11} While experienced researchers are able to minimize the possibility of injury to the laboratory animal, the stress induced by the manipulation and insertion of the tubing cannot be ignored.¹²

Several pre-clinical studies have been conducted using K14HPV16 mice,¹³ a transgenic murine model which expresses the early region genes of the human papilloma virus type 16, featuring lesions that closely resemble those of human papillomavirus (HPV) infection.^{14,15} Considering the highly stress-prone nature of this model, we aimed to develop an effective method for the oral administration of water or oil-soluble compounds that minimizes handling-induced stress in laboratory mice.

Methods

Animals

The generation of K14-HPV16 mice on a FVB/n background was previously described.¹⁶ K14-HPV16 mice were kindly donated by Drs Jeffrey Arbeit and Douglas Hanahan (University of California) through the USA National Cancer Institute Mouse Repository. The study was approved by the University de Trás-os-Montes and Alto Douro Ethics Committee (approval number 10/2013) and the Portuguese Veterinary Directorate (approval number 0421/000/000/2014). Animals were maintained in groups of four or five animals *per* cage (1284L Eurostandard Type II L, Tecniplast, Italy) in a ventilated room. They were bred according to the Portuguese (Decree-Law 113/2013) and European (Directive 2010/63/EU) legislation and maintained under controlled conditions of temperature ($23 \pm 2^\circ\text{C}$), light–dark cycle (12h light/12h dark), and relative humidity ($50 \pm 10\%$), using corncob for bedding and plastic tubes as environmental enrichment. Cages were cleaned once a week, and water was changed weekly. All animals had free access to a basic standard diet (4RF21, Mucedola, Italy) and tap water was supplied through capped water bottles *ad libitum* throughout the study. Animals' body weight, food, and water were weighed once a week using a top-loading scale (KERN® PLT 6200-2A, Dias de Sousa SA, Alcochete, Portugal). The food and water consumption for each cage was calculated by subtracting the weight of the food container or water bottle at the end of the week from its weight at the beginning of the

week. The general health status of all mice was monitored once a day for signs and symptoms of toxicity. For this, the following indicators of animal welfare were assessed: body condition (well, under conditioned, or emaciated), alertness state (normal, lethargic, stupor, moribund), response to external stimuli (normal, mild, moderate, or violent), posture (normal or changed), mucosal (normal, mild, moderate, or severe), and coat and grooming (normal, lack of grooming, rough coat, piloerection).

Genotyping

Animals were genotyped at weaning, using tail-tip samples as previously described.^{13,17} Briefly, nucleic acids were extracted, and DNA quality and purity were assessed. HPV16-E6 and E2 genes were amplified to confirm the presence of HPV DNA and a fragment of mouse β -globin was also amplified and endogenous control. Lengths of the amplicons were confirmed by agarose gel electrophoresis. Only hemizygous females were used for the transgenic groups of the experiment.

Experiments

Thirty-four female mice at four weeks of age were used. Animals were allocated to three groups: group I ($n = 15$) – wildtype mice not treated; group II ($n = 9$) – K14-HPV16 mice not treated; and group III ($n = 10$) – K14-HPV16 mice treated with celecoxib. All animals received a piece of cookie, five times a week, for four consecutive weeks. Animals from group III were treated with celecoxib at a dose of 2.5 mg/mouse/day. Each week, 125.0 mg celecoxib were dissolved in 10:90 (v/v) ethanol/distilled water and the individual 2.5 mg dose (μL ethanol) was added to a standard wheat cookie. Administration was performed by isolating each animal in a small cage, to absorb urine and avoid soiling the cookie. The animals were under permanent observation by a single researcher until completely consuming the cookie. Upon consumption, the animals were returned to their cages. During the experiment, the animals' body weight, food, and water consumption were assessed once a week using a scale. The study lasted four weeks, beginning at the time of the first cookie administration and ending when the animals were humanely euthanized at eight weeks old by intraperitoneal pentobarbital overdose, followed by exsanguination by cardiac puncture.

Statistical analysis

Data were statistically analysed with Statistical Package for Social Sciences (SPSS) version 25 (Chicago, IL, USA) using ANOVA with the Bonferroni correction for multiple comparisons. Data are presented as

mean \pm standard deviation. *P*-values lower than 0.05 were considered statistically significant.

Results and discussion

Oral gavage is the most straightforward approach to achieve precise enteric administration in rodents, but it is associated with potential adverse consequences.^{12,18} Although the animal stress and mortality related to oral gavage can be minimized when the procedure is carried out by an experienced researcher, this procedure requires animals' restraint, which may cause systemic stress response and consequently increase blood pressure, heart rate, and blood glucocorticoid levels.^{19,20} Stress may influence animal behaviour and confound experimental results. In some cases, serious complications have also been associated with oral

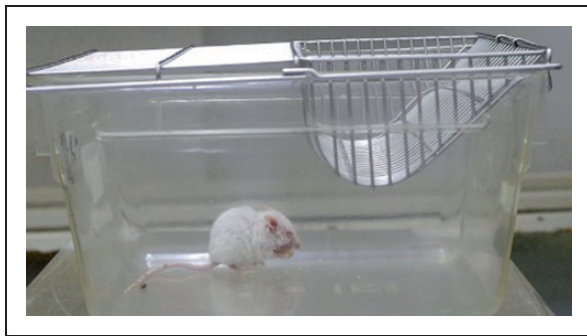


Figure 1. K14HPV16 mouse ingesting the cookie fragment. Single frame from a video recording, with the mouse fully consuming the cookie fragment within 15 seconds.

gavage, including reflux, tracheal administration, aspiration, trauma, oesophageal irritation and perforation, pneumonia, and death.^{9,12,21–27}

Our approach was to take advantage of the normal feeding behaviour of mice. Several food products were considered as candidate vehicles for the incorporation of drugs of interest in them and tested alone to assess their palatability. Of these, the most promising one was an easily obtainable, affordable, and commercially available variety of standard wheat cookie.²⁸ The cookie was found to be highly palatable, capable of holding structural integrity while being manipulated by the mice. A conditioning effect was also observed in which animals took progressively less time to find the fragment and consume it. In the first week the animals took 5–10 minutes to consume the cookie, and the consuming time decreased overtime. In the last week of the experiment, the cookie was immediately found and consumed, taking less than one minute. No changes in the indicators of animal welfare were observed.

Measuring approximately 5.0 cm in diameter and 0.4 cm in thickness, with a weight of about 6.3 g *per* unit, these cookies have a porous structure that allows the retention of a certain amount of fluids without compromising structural integrity. For standardizing purposes, the cookies were manually broken up into uniformly sized fragments ($150 \text{ mg} \pm 10\%$), which were then used to retain commonly used solvents. The cookie's energy value is 17.94 KJ *per* gram ($\sim 270 \text{ J per}$ dosing fragment), which was considered to have a negligible effect on energy intake, even

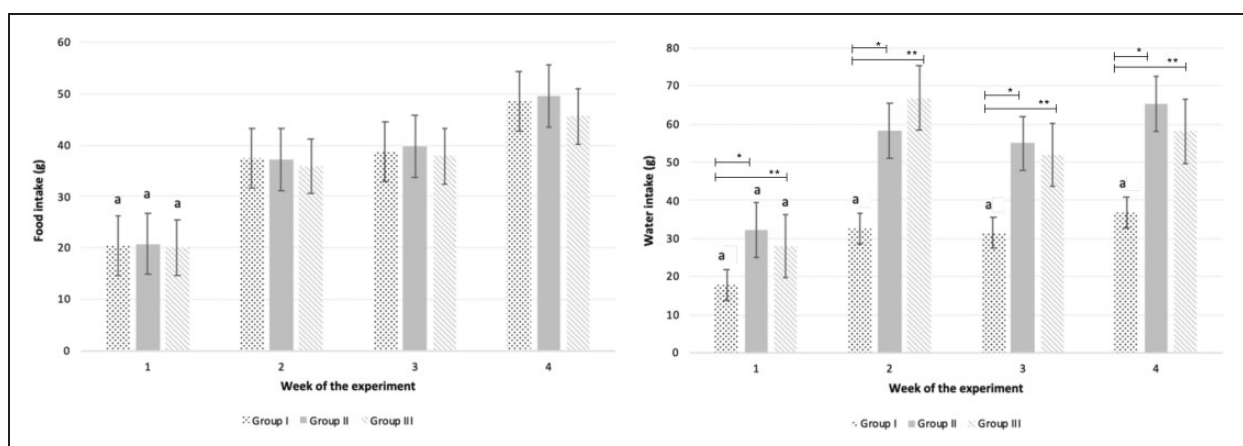


Figure 2. Variation of food and water intake *per* animal *per* week was addressed throughout the study in all experimental groups (group I – wildtype not treated; group II – K14-HPV16 not treated; group III – K14-HPV16 treated with celecoxib). ^aDifferent from weeks 2, 3, and 4. Differences between groups are represented by horizontal lines.

*Different from group II.

**Different from group III ($p < 0.05$).

Data are presented as mean \pm standard error of mean.

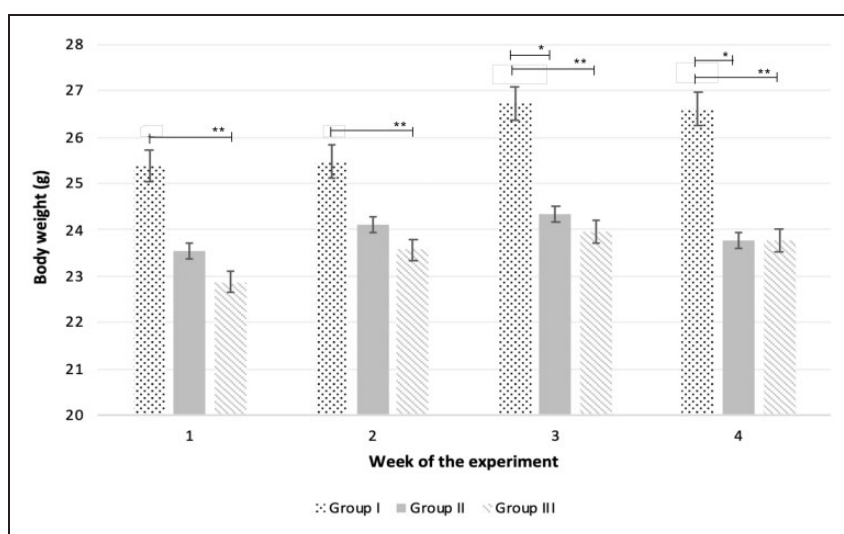


Figure 3. Variation of body weight throughout the study in all experimental groups (group I – wildtype not treated; group II – K14HPV16 not treated; group III – K14HPV16 treated with celecoxib). Differences between groups are represented by horizontal lines.

*Different from group II.

**Different from group III ($p < 0.05$).

Data are presented as mean \pm standard error of mean.

assuming a daily administration.²⁹ The proposed solvents (distilled water or ethanol) were applied to the fragments by pipetting a maximum of 1 μ l/mg, which allowed the fragment to remain solid, and dried overnight in an oven, at 40°C before being administered to the animals. Care is needed to apply the solvent slowly, ensuring that it is absorbed and does not fall off the surface. For heat-sensitive drugs, drying the cookies at room temperature may be advisable. Additionally, the impact of the cookie on pharmacokinetic parameters for each test compound may vary and its interference with celecoxib was not tested in the present study.

The administration by isolating each animal in a small cage allowed direct observation of complete consumption of the fragment (Figure 1). The technique allowed near simultaneous administration of the fragment to large numbers of individuals (up to 30 animals) in a very short time span, providing considerable time savings over gavage. Over 80% of the animals readily accepted the cookie fragment, and these were the ones chosen for incorporation into studies. We found that the addition of unmedicated cookie fragments had no impact on food and water consumption for the control groups (Figure 2). The transgenic mice presented a lower body weight when compared with wildtype mice, which has been associated with their health condition (Figure 3).^{30–32}

This work was only performed in female transgenic mice, which may constitute a limitation. More studies

addressing the feed behaviour in males and in other mice models are warranted.

Conclusions

This method allows for the fast and efficient oral administration of compounds to mice, while inducing very little stress with animal manipulation. It constitutes a promising alternative to oral gavage in pre-clinical studies with laboratory mice.

Declaration of conflicting interests


The authors have no conflicts of interest to declare.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the Research Center of the Portuguese Institute of Oncology of Porto (CI-IPOP 37-2016), by project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy), projects POCI-01-0145-FEDER-006958 and UID/AGR/04033/2020, funded by FEDER funds through COMPETE2020 – Operational Competitiveness and Internationalization Program (POCI) – and by national funds through the Portuguese Foundation for Science and Technology (FCT). Rui M Gil da Costa received a grant from FCT, funded by the Portuguese Government and the Social European Fund (grant number SFRH/BPD/85462/2012).

ORCID iDs

T Neto  <https://orcid.org/0000-0002-1406-414X>

AI Faustino-Rocha  <https://orcid.org/0000-0001-5572-6317>

References

- Gregory SG, Sekhon M, Schein J, et al. A physical map of the mouse genome. *Nature* 2002; 418: 743–750.
- Adam TC and Epel ES. Stress, eating and the reward system. *Physiol Behav* 2007; 91: 449–458.
- Tamashiro KL, Hegeman MA, Nguyen MM, et al. Dynamic body weight and body composition changes in response to subordination stress. *Physiol Behav* 2007; 91: 440–448.
- Tamashiro KL, Nguyen MM, Ostrander MM, et al. Social stress and recovery: implications for body weight and body composition. *Am J Physiol Regul Integr Comp Physiol* 2007; 293: 1864–1874.
- Ulrich-Lai YM and Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 2009; 10: 397–409.
- Mandillo S, Tucci V, Holter SM, et al. Reliability, robustness, and reproducibility in mouse behavioral phenotyping: a cross-laboratory study. *Physiol Genomics* 2008; 34: 243–255.
- Ueno H, Suemitsu S, Murakami S, et al. Feeding behavior of mice under different food allocation regimens. *Behav Neurol* 2019; 2019: 1581304.
- Suckow MA, Danneman P and Brayton C. *The laboratory mouse*. Boca Raton: CRC, 2001.
- Murphy SJ, Smith P, Shaivitz AB, et al. The effect of brief halothane anesthesia during daily gavage on complications and body weight in rats. *Contemp Top Lab Anim Sci* 2001; 40: 9–12.
- Fortman JD, Hewett TA and Bennet BT. *The laboratory nonhuman primate*. Boca Raton: CRC, 2002.
- Atcha Z, Rourke C, Neo AH, et al. Alternative method of oral dosing for rats. *J Am Assoc Lab Anim Sci* 2010; 49: 335–343.
- Arantes-Rodrigues R, Henriques A, Pinto-Leite R, et al. The effects of repeated oral gavage on the health of male CD-1 mice. *Lab Anim (NY)* 2012; 41: 129–134.
- Paiva I, Da Costa RMG, Ribeiro J, et al. A role for MicroRNA-155 expression in microenvironment associated to HPV-induced carcinogenesis in K14-HPV16 transgenic mice. *PLoS One* 2015; 10(1): e0116868.
- Arbeit JM, Münger K, Howley PM, et al. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. *J Virol* 1994; 68(7): 4358–4368.
- Coussens LM, Hanahan D and Arbeit JM. Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice. *Am J Pathol* 1996; 149: 1899–1917.
- Arbeit JM, Münger K, Howley PM, et al. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. *J Virol* 1994; 68: 4358–4368.
- Paiva I, Gil da Costa RM, Ribeiro J, et al. MicroRNA-21 expression and susceptibility to HPV-induced carcinogenesis – role of microenvironment in K14-HPV16 mice model. *Life Sci* 2018; 128: 8–14.
- Jones CP, Boyd KL and Wallace JM. Evaluation of mice undergoing serial oral gavage while awake or anesthetized. *J Am Assoc Lab Anim Sci* 2016; 55: 805–810.
- Dobráková M and Jurčovicová J. Corticosterone and prolactin responses to repeated handling and transfer of male rats. *Exp Clin Endocrinol* 1984; 83: 21–27.
- Kramer K, Voss HP, Grimbergen JA, et al. Telemetric monitoring of blood pressure in freely moving mice: a preliminary study. *Lab Anim* 2000; 34: 272–280.
- Brown AP, Dinger N and Levine BS. Stress produced by gavage administration in the rat. *Contemp Top Lab Anim Sci* 2000; 39: 17–21.
- Balcombe JP, Barnard ND and Sandusky C. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 2004; 43: 42–51.
- Germann PG, Ockert D and Tuch K. Oropharyngeal granulomas and tracheal cartilage degeneration in Fischer-344 rats. *Toxicol Pathol* 1995; 23: 349–355.
- Ökva K, Tamosevičiute E, Ciziute A, et al. Refinements for intragastric gavage in rats. *Scand J Lab Anim Sci* 2006; 33: 243–252.
- Eichenbaum G, Damsch S, Looszova A, et al. Impact of gavage dosing procedure and gastric content on adverse respiratory effects and mortality in rat toxicity studies. *J Appl Toxicol* 2010; 31: 342–354.
- Damsch S, Eichenbaum G, Tonelli A, et al. Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). *Toxicol Pathol* 2011; 39: 348–360.
- Walker MK, Boberga JR, Walsh MT, et al. A less stressful alternative to oral gavage for pharmacological and toxicological studies in mice. *Toxicol Appl Pharmacol* 2012; 260: 65–69.
- Pereira DFC, Guiné R and Correia P. Estudo comparativo de bolacha Maria de diferentes marcas. Instituto Politécnico de Viseu, Escola Superior Agrária, Viseu, Portugal, 2012.
- Vieira de Castro. Bolachas Maria. <https://vieiradecastro.pt/collections/dia-a-dia/products/bolachas-maria-300g>. Accessed in: 26th July 2021.
- Santos C, Ferreira P, Sousa H, et al. Ptaquiloside from bracken (*Pteridium* spp.) inhibits tumour-infiltrating CD8+ T cells in HPV-16 transgenic mice. *Food Chem Toxicol* 2016; 97: 277–285.
- Santos C, Neto T, Ferreira P, et al. Celecoxib promotes degranulation of CD8+ T cells in HPV-induced lesions of K14-HPV16 transgenic mice. *Life Sci* 2016; 157: 67–73.
- Gil da Costa RM, Aragão S, Moutinho M, et al. HPV16 induces a wasting syndrome in transgenic mice: amelioration by dietary polyphenols via NF- κ B inhibition. *Life Sci* 2017; 169: 11–19.

Méthode rapide et de faible intensité pour l'administration orale à un grand nombre de souris: alternative possible au gavage oral

Résumé

L'administration orale de médicaments aux animaux de laboratoire est une cause de stress important. Lorsqu'elle est associée à des animaux qui sont déjà dans des circonstances difficiles en raison de la maladie modélisée, il existe un risque important d'augmentation de la morbidité et de la mortalité, ce qui influence les résultats. Face à ces contraintes, une méthode d'administration orale de faible intensité a été mise au point, basée uniquement sur le comportement naturel des animaux et sur un conditionnement minimal. Des doses précises de médicaments ont été administrées dans un fragment de biscuit de blé standard disponible localement, fournissant à la fois un véhicule agréable et une matrice absorbante pour le médicament. L'administration rapide à un grand nombre d'animaux a donc été réalisée, assurant ainsi le bien-être des animaux et la facilité de manipulation. Cette méthode constitue une alternative prometteuse au gavage oral dans les études précliniques de médicaments menées sur des souris de laboratoire.

Schnelle und wenig belastende Methode zur oralen Verabreichung an eine große Anzahl von Mäusen – eine mögliche Alternative zur Schlundsonde

Abstract

Die orale Verabreichung von Medikamenten an Versuchstiere ist mit erheblichem Stress verbunden. Tiere, die aufgrund der zu modellierenden Krankheit bereits unter Belastung stehen, werden durch diesen zusätzlichen Stress einem erheblichen Risiko für erhöhte Morbidität und Mortalität ausgesetzt, was auch Auswirkungen auf die Versuchsergebnisse nach sich zieht. Angesichts dieser Umstände wurde eine Methode zur oralen Verabreichung mit geringer Belastung entwickelt, die ausschließlich auf dem natürlichen Verhalten der Tiere und einer minimalen Konditionierung basiert und bei der präzise Dosierungen der Medikamente in einem Stück örtlich verfügbaren, marktüblichen Weizenkeks verabreicht werden, der sowohl ein wohlschmeckendes Vehikel als auch eine absorbierende Matrix für das Medikament darstellt. Auf diese Weise wurde eine schnelle Verabreichung an eine große Anzahl von Tieren erreicht, wobei das Wohlergehen der Tiere gewahrt und eine einfache Handhabung gewährleistet wurde. Diese Methode ist eine vielversprechende Alternative zur oralen Schlundsonde in präklinischen Medikamentenstudien mit Labormäusen.

Un método rápido y de baja intensidad para la administración oral para grandes números de roedores: una posible alternativa a la sonda oral

Resumen

La administración oral de medicamentos en animales de experimentación es algo que les provoca gran estrés. Cuando esto se suma a animales que ya están pasando por un momento extenuante debido al desarrollo de la enfermedad, existe un riesgo significativo de aumentar la mortalidad y la morbilidad, influyendo así en los resultados. Con estos problemas en mente, se creó un método de baja intensidad para la administración oral basado únicamente en el comportamiento natural de los animales y un acondicionamiento mínimo, en el que dosis precisas de medicación se administraban en un fragmento de galleta de trigo estándar disponible localmente, lo que ofrecía un vehículo sabroso y una base absorbente para el medicamento. De este modo se consiguió una administración rápida para un gran número de animales, protegiendo así el bienestar de los mismos y garantizando una manipulación sencilla. Este método es una alternativa prometedora a la sonda oral para estudios de medicamentos preclínicos con ratones de laboratorio.