Video Article

Utilizing an Orally Dissolving Strip for Pharmacological and Toxicological Studies: A Simple and Humane Alternative to Oral Gavage for Animals

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Abstract

Prior to testing novel therapeutics in humans, short and long term preclinical (*i.e.*, animal), repetitive pharmacological and toxicological testing is required. In most cases, the preferred route of administration is via oral delivery. At the present time, oral delivery is mostly accomplished using an oral gavage procedure, in part, because it can achieve consistent and precise dosing in the animal model. Although this method is well established it does have complications that can result in a high rate of animal attrition. To this end, the procedure introduced here describes an alternative to the oral gavage method in which the desired drug is incorporated into a tastant, orally dissolving strip (ODS) that can simply be presented to the test animal where it is then rapidly taken up with minimal manipulation of the test subject. Herein, we demonstrate that preclinical, oral drug delivery using the ODS method represents a safe, convenient, and humane alternative to oral gavage.

Video Link

The video component of this article can be found at https://www.jove.com/video/53770/

Introduction

In order for the successful translation of a drug from preclinical (animal testing) to clinical development, a series of well-defined, acute and chronic pharmacological and toxicological studies need to be performed in animal models using the intended clinical route of administration (which is normally the oral route). To accomplish this goal, most pre-clinical studies currently utilize an oral gavage procedure given that this method results in a consistent and precise delivery of the test compound to the animal. However, in many instances, oral gavage is not well tolerated by the animal and accumulating evidence suggests that the method is associated with a significant amount of stress induction[1].

This is especially true in the development of treatments for chronic life-long conditions that require longer-term preclinical studies, such as is the case with, but not limited to, nearly every neuropsychiatric disorder. For instance, in a transgenic mouse model of Alzheimer's disease, it was recently suggested that the gavage method itself could confound experimental results due to dosing induced stress[2]. Similarly, in a mouse model of alcohol consumption, unpublished findings from our own laboratory have found that the repetitive insertion of the gavage tube - in and of itself - can significantly reduce the level of alcohol intake to the point of jeopardizing the integrity of the experimental paradigm (see **Figure 1**).

Given the aforementioned limitations associated with the oral gavage procedure, considerable effort has been put towards developing novel methods of preclinical oral drug delivery. Current alternative approaches include incorporating the test compound into peanut butter mixtures[3], gelatinous molds[4-5], chocolate pellets[6], drinking water[7], and wafer crackers[8], all of which are associated with varying degrees of issues including the inability to be used reliably in the animal model, lack of adoption of test compound uptake by the test subject or individual preference for flavor that makes the drug less readily consumed[9].

The procedure introduced herein describes a method in which the desired drug is incorporated into a flavored, orally dissolving strip (ODS) that can be easily and readily be used to orally administer the test compound to the test animal. This paper focuses on demonstrating the effectiveness of the ODS method using mice. However, there is no reason to expect that the same method would not also be useful in other rodent as well as larger mammalian subjects. Clinically, the use of ODS is starting to be adopted in pediatric and geriatric patients, as a way to overcome swallowing difficulties[10]. We propose that ODS can also be used successfully in preclinical drug discovery programs as a safe, convenient, and humane alternative to oral gavage.

Protocol

All procedure described have been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California.

1. Animal Handling and Habituation

1. Introduction
    1. Allow mice to acclimate to the husbandry conditions and handling technique of the experiment to decrease stress during the ensuing drug administration procedures. During this time, record the body weight and food intake of each mouse; if desired, they can later be used as a non-invasive point of comparison to assess drug toxicity (optional). The former can also be used to determine weight-based drug dosing.
    Note: Anecdotally, the length of the habituation period should be determined based on the number of experimenters interacting with the animal. In our experience, mice of the C57BL/6J strain have been observed to have trouble habituating to handling by more than ~5 people within our typical week-long habituation period. If feasible, experimenter(s) are also encouraged to refrain from using scented lotions, soaps, and perfumes before handling the animals as previous reports indicate novel stimuli, such as smells, may hinder the habituation period[11].

2. Steps
    1. Beginning at least 5 days prior to the initiation of the study, transfer the animals to the room in which the subsequent experiments will occur.
    2. Record the weight of the mouse by firmly grasping the middle of the tail and gently lifting the animal out of the cage and into the weight boat.
        1. Place the mouse on the scale as soon as it is lifted out of the cagekeeping in mind to properly grasp the medial/proximal aspect of the tail, as to avoid degloving injuries, which can occur when grasping the distal aspect of the tail[12] .
        2. Record the weight value once the mouse has been safely placed into the weigh boat.
    3. Record the weight of the food for a recommended 2 consecutive days, in order to establish baseline food consumption levels that will help capture potential toxicities during the experiment[13].

2. Preparation of Feeding Needle, Sucrose Solution, and Individual ODS

1. Use an autoclave to sterilize the stainless steel gavage needles in preparation for the experiment. Prepare 2 needles/group and 1 additional needle to be used as a back-up if need be.
Note: This study utilized a 1.5 inches, curved, 18 G, stainless steel feeding needle with a 2.25 mm ball at the end of the needle.
2. Add 20 ml of water to 0.85 g of sucrose to create a 4.25% (w/v) sucrose solution.
Note: Depending on the parameters under assessment other low doses of sucrose could potentially also be used[14].
3. Prepare the oral dissolving strip (ODS) for each animal by cutting the standard rectangular test strip into a piece that is 0.5 cm inches in diameter using a commercially available single quarter inch hole-puncher. The rectangular test strip used in this demonstration was formulated to contain 6 mg of ivermectin (IVM), and when cut to this size, allocates a 0.21 mg dosage to each individual circular piece approximating a 10 mg/kg dose for each animal (our desired dose/animal). This dose can be adjusted by concentrating/diluting the overall formulation on the rectangular strip and/or cutting the strip into different size.

3. Drug – Delivery

1. Record the weight values as previously described in steps 1.1-1.2.
2. After recording the weight, move the mouse to the metal grid cage top. Once there, using the dominant thumb, index finger, and 3rd finger, gently pull back on the tail. This will cause the mouse to inadvertently grip the wire cage top and stretch out its back, stretching its body away from the experimenter.
3. Grasp the scruff of the neck with the non-dominant thumb, index finger, and 3rd finger to comfortably restrain the animal with a grip that is secure.
Note: An experimenter with a smaller hand can also use the 4th finger, of the same hand, to pull back the scruff of the back and obtain a more secure hold of the mouse. Additionally, depending on individual preference, ring finger, and/or pinky finger may also be used to secure the tail however this is not necessary[12].
4. Hold the mouse in an upright position.
5. Caution: Observe the color of the mucosal membranes of the mouse. A purple/blue appearance is an indication that the experimenter is cinching the scruff too tightly and the mouse cannot breathe.
    1. If this occurs, immediately put the mouse back down on the metal cage top and allow it to rest for ~2-3 min before reattempting to restrain the animal.
6. Submerge the bulb tip of the gavage needle in 4.25% sucrose solution to serve as a tastant adhesive to the ODS.
7. Press down on a pre-cut ODS with the bulb tip of the gavage needle to attach the orally dissolving strip.
8. Present the ODS to the mouse by placing it near the nostrils and/or mouth, and allow the animal to consume the film. Alternatively the ODS can be delivered topically to the oral cavity by placing the thin strip directly onto the tongue for the mice to swallow.
9. Replace the needle mid-group during the drug-dosing session to prevent the spread of infection.

Representative Results

In the following representative investigations, social drinking was modeled using a 24 hr two-bottle choice (TBC) paradigm as previously described[15]. Briefly, mice had access to two bottles of solution, one of which contained water, and the other a 10% (v/v) ethanol solution. Subjects were subsequently assigned to drug treatment groups so that the average 10E (10% ethanol/90% water v/v) intake was similar across groups.

Figure 1 illustrates the effects of ivermectin (IVM) on preference drinking when orally administered using a gavage vehicle. This pilot study had 3 groups: N/A, placebo, and IVM representing no gavage, corn oil (vehicle) gavage, and IVM (10 mg/kg, diluted in corn oil) gavage respectively. Mice were treated for 10 days, 5 consecutive days per week for 2 weeks. As can be see below, IVM was able to significantly reduce 10E intake across the study when compared to the placebo group. Compared to N/A, the ethanol intake values of the placebo group (~5 g/kg), are much too low (48% lower than the non-gavaged N/A group), and thus, may not be an accurate representation, or "model", of social drinking behavior (~10-15 g/kg). Meaning that an unknown factor associated with the gavage procedure managed to jeopardize the integrity of the drinking paradigm.

Figure 2 illustrates the same experiment subsequently performed utilizing the ODS delivery system[15]. The study had 3 groups: 1) no oral film, 2) key lime flavored oral film with no IVM and 3) key lime flavored oral film with IVM (0.21 mg, which is equivalent to the 10 mg/kg administered in the aforementioned study) referred to as control, placebo and IVM, respectively. Mice were treated for 20 days, 5 consecutive days per week for 4 weeks. There was no significant difference in ethanol intake between the N/A and placebo treated mice, indicating that the ODS placebo group represented a valid control with which a comparison to the IVM group could then be made. In agreement with previous work[13,15], IVM significantly and consistently reduced 10E intake.

A pictorial representation of the technique is shown in **Figure 3**.

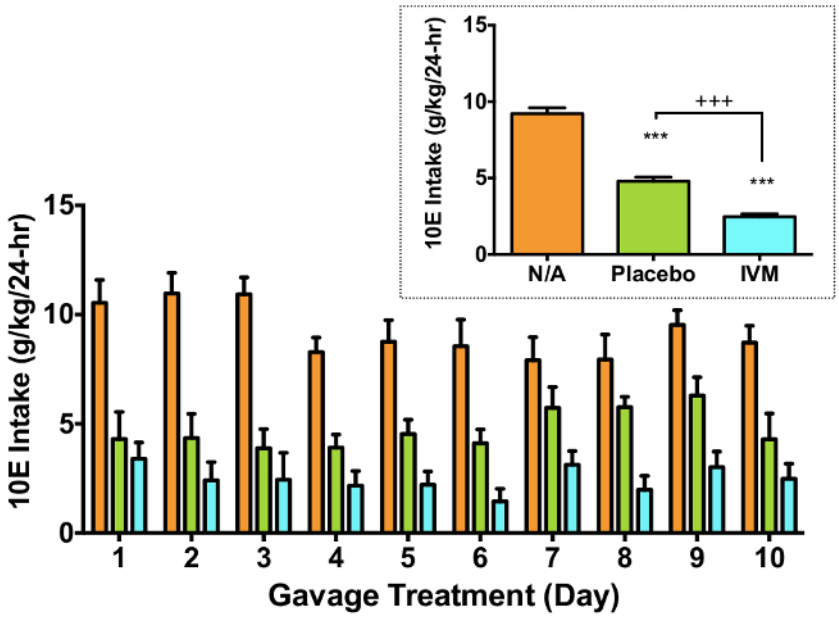

Figure 1. Gavaged Control Mice are Unable to Maintain Ethanol Intake Levels Representative of the 24 hr TBC Paradigm. Male C57BL/6J mice were treated for 10 days (5 consecutive days per week for 2 weeks). Vehicle gavage treatment of the placebo group significantly altered ethanol intake values by 48% when compared to the non-gavage N/A group. Although IVM significantly reduced 10E intake compared to placebo, the low 10E intake values of the placebo group added a second confound to the study, which could have the effect of compromising the usefulness of the group as an adequate drinking control for the study. Inset graphs illustrate the average 10E intake over the 10 day treatment. Values represent the mean ± SEM for n = 6-8 mice/group. +++*P* <0.001 versus Placebo, and ****P* <0.001 versus N/A, Turkey Post-Hoc Test. Please click here to view a larger version of this figure.

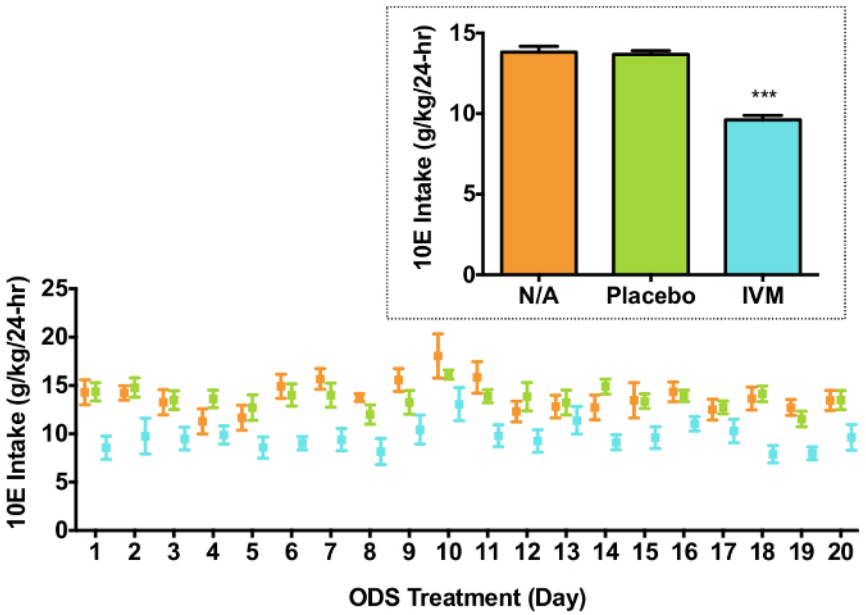

Figure 2. ODS Treatment of IVM. Female C57BL/6J mice were treated for 20 days (5 consecutive days per week for 4 weeks). Placebo ODS treatment had no significant effect on 10E intake, relative to the non-treated N/A group, maintaining the validity of placebo ODS as an adequate social drinking control. IVM significantly reduced 10E intake compared to placebo control. Inset graphs illustrate the average 10E intake over the 20 day treatment. Values represent the mean ± SEM for n = 6-8 mice/group. ****P* <0.001 versus Placebo, Turkey Post-Hoc Test.. This figure has been modified from Yardley *et al.*[15] Please click here to view a larger version of this figure.

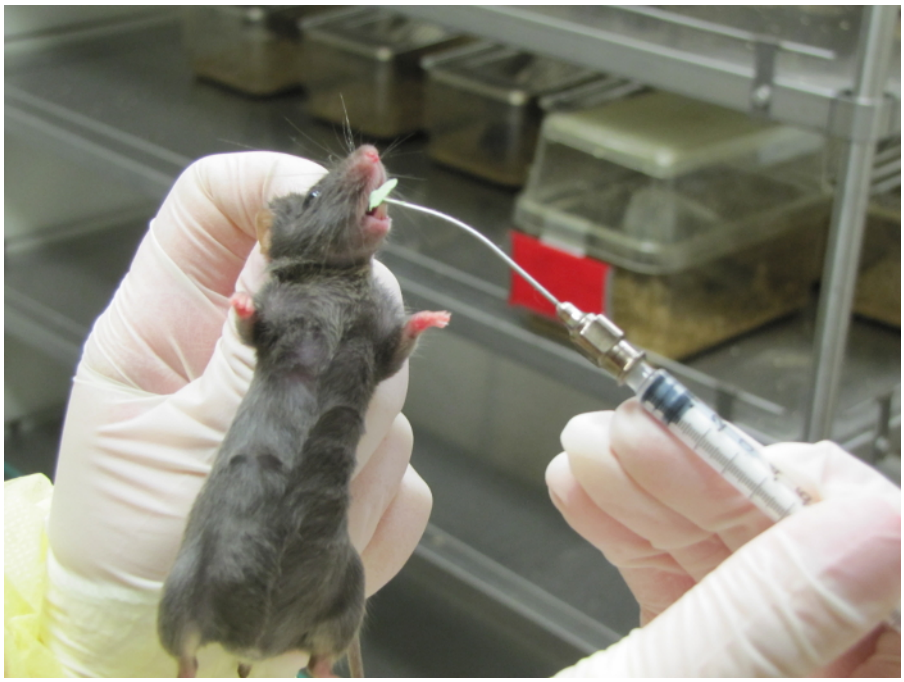

Figure 3. Pictorial Representation of ODS Delivery. Photographic depiction of the procedure. Please click here to view a larger version of this figure.

Discussion

Oral gavage is associated with numerous complications that result in potentially compromised data collection and high rates of animal attrition[1]. Here a simple method of oral drug delivery is introduced through which mice (and potentially other animals) can easily and reliably consume the drug of choice through an orally dissolving strip (ODS). Notably, the use of this method represents a safe, convenient, and humane alternative to oral gavage.

Other current alternatives to oral gavage, although somewhat promising, are associated with various limitations that preclude them from covering the full range of needs associated with drug-dosing studies such as an inability to be used in mice, or individual preference for flavor that make

the drug less readily consumed[9]. In contrast, ODS can be formulated in a wide-variety of flavors which is important as individual preferences for flavor can change over time and flavor preferences may differ across various strains and species.

Proper animal handling is an essential component of this technique. Highlighting the importance of the habituation period, it should be noted that a given mouse may be harder to restrain if it is particularly young or unaccustomed to handling. Any difficulty grasping the mouse should subside over time as the animal is familiarized to handling and becomes less fearful of the experimenter(s). The C57 mouse strain exemplified in the representative results has been observed to habituate to handling procedures relatively quickly (within roughly 1-3 handling sessions). Although this duration may defer across various species and strains, there is no reason to assume the acclimation time-frame would escalate significantly. Novice technicians working with rodents should be cognizant that, when suspended and dangling in the air for more than a few seconds, mice are susceptible to the development of a degloving injury to the tail[12]. The chances of this occurring increase with the weight of the mouse as well as with the positioning of the hand, with a grasp closer to the tip of the tail more likely to cause an injury. Specific to the scope of this protocol, it is imperative that the mouse is placed on the metal grid cage top, or in an alternative apparatus, when being transferred from cage to scale, as well as in preparation for drug-delivery. Restraint during the drug-delivery process is another critical step within the protocol. ODS consumption can occur by placing the thin film near the nostrils and/or mouth of the mouse, in which case the subject will lean forward to consume the film. Alternatively the ODS can be delivered topically to the oral cavity by manually placing the film directly onto the tongue for the mouse to swallow.

Investigations that involve the assessment of stress-sensitive parameters can be confounded by the use of the standard manual restraint techniques depicted in this protocol and simple adjustments to the methodology can easily be made to accommodate studies in which restraint is prohibited. In this modified approach, the ODS could be placed directly in the cage for the animal to consume. Observations gleaned from pilot studies in our own lab indicate that this would best be performed by placing the animals in a cage without any bedding/nesting material, just for the duration of the drug delivery procedure, in order to increase the ease with which ODS consumption can be visually monitored by the experimenter(s). Moreover, it might be appropriate to initiate a training period during which time the animals are introduced to the ODS so that they can overcome the initial innate avoidance of novel food[4]. Of course further studies would be needed to optimize a restraint-free protocol for ODS delivery, however there is no reason to believe that such a procedure could not be done with ease.

There are some limitations to the ODS method. For instance, given that the ODS are a fast dissolving bio-adhesive polymer, the experimenter will need to consider issues of absorption and metabolism in that depending on the formulation of the strip, some of the drug could potentially bypass first pass metabolism due to a portion of the drug being absorbed via lingual or buccal mechanism. On the other hand, for CNS targeted drugs, lingual or buccal absorption could be a benefit allowing more of the compound to reach the CNS target prior to metabolism. Overall, this issue can be addressed by changing the formulation of the ODS that will result in the drug either not being readily available until it is absorbed via the gut or to take advantage of direct access to the CNS. Additionally, mice may develop conditioned taste aversion and withdraw from voluntary ODS consumption if the drug elicits aversive side effects. Although we have not observed this in our studies, one potential method to ameliorate such a situation would be to alternate the flavor of the test strip to help keep a bit of novelty for the test animal. Lastly, at high concentrations the ODS might not be palatable. However, this issue could also be addressed by changes in formulation where the drug itself is encapsulated and thus taste would no longer be an issue, or alternatively, by simply administering different flavored strips, so that there remains novelty in the flavor for the test subject.

Notably, in terms of the data reproducibility, ODS can be formulated to adhere to the tongue or cheek of the animal and designed for rapid oral disintegration to quickly release the drug, thus assuring proper dosing is achieved. And, with ODS administration, the need for gavage and other unpleasant invasive experiences for the animal is eliminated. Not only is this more humane but it also minimizes the effects of stress as a variable in the experiment. It should be noted that the ODS method can be further optimized, as in many new manufacturing processes, which may further improve the pharmacokinetics of the test compound as ODS is expanded for additional therapies. One limitation that should be pointed out regards the fact that the development of ODS is in the early stages of development. Issues concerning the loading of different physicochemical characteristic drugs over the tastant/ODS platform remains a challenge to the versatility and generalization of this technique. This is an area of ongoing investigation.

The proposed technique is a simple and novel technique that can be adopted for use in short and long term preclinical animal studies and for the delivery of medicines to animals (*e.g.*, dogs). Drug delivery through ODS represents a new, fast, convenient and humane approach compared to the existing traditional approaches like tablets, capsules, liquids, powders, and gels and, oral gavage.

Disclosures

DD has applied for a patent as a Co-Inventor on this method and is interested in sharing this method with the scientific committee to make this technique well known and readily available for chronic animal testing (**Oral Delivery of Drug Actives in Laboratory Animals Using Fast-Dissolving Oral Films** (Davies, Co-Inventor, Patent Application filed 6-23-2014).

No other competing financial interests are noted.

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