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A novel approach for long-term oral drug administration in animal research

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ABSTRACT

In the field of pharmacological research, the oral consumption of anastrozole, an aromatase inhibitor, when added to an animal's drinking water is hindered by poor drug palatability and environmental loss of drug solution. To overcome these caveats, we developed a novel approach for the oral delivery of anastrozole mixed in a solid hydration gel matrix that functions as a replacement for water. Heated hydration gel was mixed with anastrozole and distributed into a gel delivery device consisting of a 50 mL plastic conical tube containing four stacked 200 μL pipette tips to allow for air pressure induced gel disbursement. Transgenic female 3xTgAD mice were randomized to receive either anastrozole-treated or untreated hydration gel at 3 months of age. Body weights were recorded weekly, and gel consumption was measured every 1–3 days. Six months post treatment mice were killed and serum anastrozole levels were determined using liquid chromatography–mass spectrometry (LC–MS). Anastrozole-treated mice gained significantly more weight despite consuming significantly less hydration gel compared to vehicle treated mice. LC–MS analysis, using a low serum volume (10 μL), revealed average anastrozole serum levels of 2.91 ng/mL. Anastrozole-treated ovarian tissue displayed ovarian cysts, massive edema-like stroma, and also lacked corpa lutea compared to control mice. These findings demonstrate that hydration gel delivered using the newly developed oral delivery method is a viable approach for pharmacological research involving compounds with poor palatability, low water solubility, and cost prohibitive compounds where environmental loss needs to be minimized.

1. Introduction

Long-term oral drug administration in rodents has several methodological challenges including minimizing stress to the animal, drug palatability, and in the case of liquids, reducing the amount of drug lost due to water bottles that leaked, and bedding contamination. The use of anastrozole, an aromatase inhibitor, which interrupts a critical step in the body's synthesis of estrogen (Plourde et al., 1994) and is used orally in breast cancer treatment for post-menopausal women (Howell et al., 2005), suffers from many of these methodological caveats. Anastrozole has sparse water solubility (Sarkar and Yang, 2008) and displays poor palatability (Gary Nunn, AstraZeneca, personal communication). In studies where rodents were given water bottles containing anastrozole for three to twelve months (Beaton and deCatanzaro, 2005; Turner et al., 2000), leakage made dose calculations difficult (Beaton and deCatanzaro, 2005). While dosing animals using a gavage syringe provides a specific drug dose and overcomes palatability issues and drug loss, oral gavage is stressful particularly when studies require daily administration over weeks to months (Balcombe et al., 2004). Therefore, investigators will often administer poorly tolerated drugs using alternate administration routes such as subcutaneous to avoid the stressful nature of gavage dosing, or intracerebrally to ensure presence in the brain. However, these in vivo dosing methods often differ from the route of administration used for humans thereby limiting the applicability of these findings to the human condition. Here we describe the development of a novel oral drug delivery device that combines anastrozole with a thermo-reversible hydration gel housed in a gel delivery device, which overcomes these technical problems.

Thermo-reversible hydration gel contains >92% water and when heated transforms into a semi-liquid. When cooled to room temperature, the gel reverts back to its original solid state. Although hydration gels are available in various flavors to mask the taste of compounds with poor palatability, delivering medicated hydration gel in a manner that prevents rodents from playing with, or otherwise contaminating the gel, remains a challenge. To overcome these issues, we developed a novel gel delivery device that provides sufficient amounts of gel to hydrate/treat a cage of five mice

Abbreviations: %RSD, % relative standard deviation; ESI, electrospray ionization; LLOD, lower limit of detection; LLOQ, lower limit of quantification; MRM, multiple reaction monitoring; QCs, quality controls.

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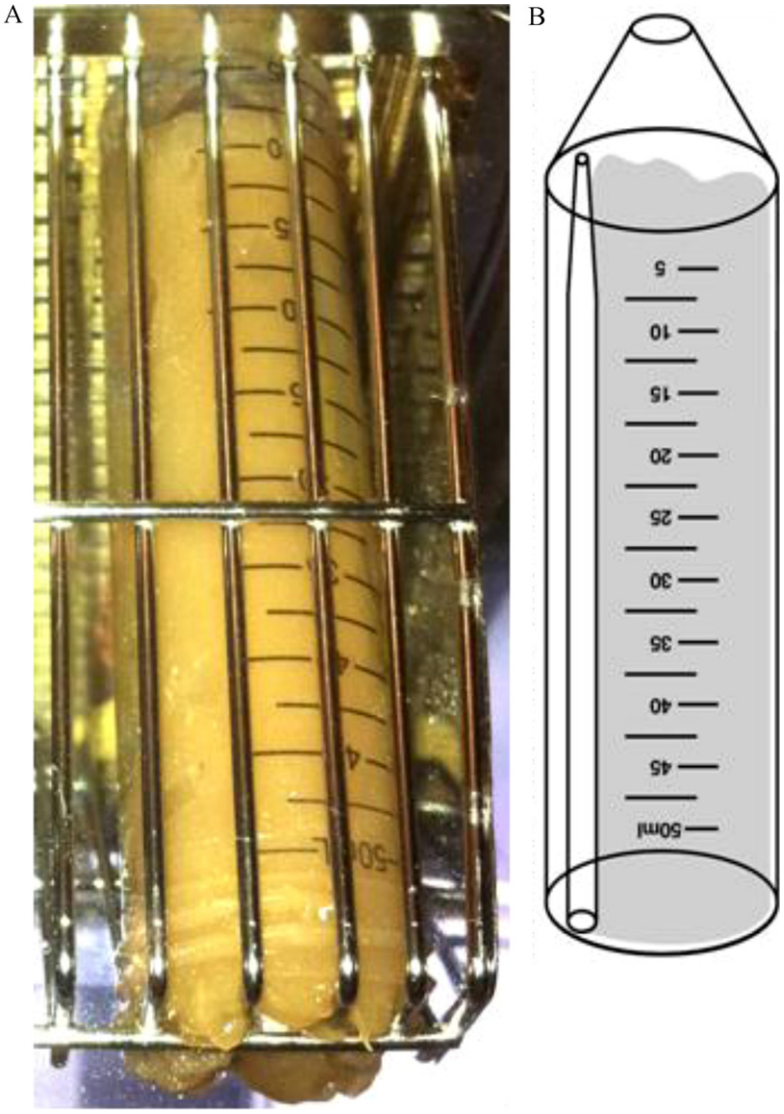

Fig. 1. (A) Photograph showing placement of the gel delivery device within the cage lid. (B) Schematic drawing of the 50 mL inverted conical tube containing 2 mL stereological pipette to create an air space between the inner wall of the plastic conical tube and the gel, which allows the gel to slide down during consumption. Milliliter gradations enable quantification of gel consumption.

for up to five days, protects against gel evaporation and bedding contamination, while allowing recording of gel consumption and reduces the time spent by investigators dosing mice.

2. Material and methods

2.1. Animals

Three-month old female 3xTgAD mice, which over expresses the familial Alzheimer's disease (FAD) genes APP_{SWE}, $PS1_{M146V}$, and Tau_{P301L} (Oddo et al., 2003), were generated from breeding pairs provided by Dr. Frank LaFerla from the University of California Irvine. Mice were housed four to five per plastic cage and maintained under constant room temperature and humidity on a 12:12-h light:dark cycle (lights on at 6 am). Littermates were randomized to receive either anastrozole-treated or untreated hydration gel (N = 8–9/group) as well as food pellets (2020×; Teklad, Indianapolis, IN), ad libitum. Cages were changed twice a week. Body weights were recorded weekly, and gel consumption was collected every 1–3 days. Vaginal cytology was used to identify the proestrus phase during the last week of treatment (Goldman et al., 2007). Mice in proestrus were deeply anesthetized using a mixture of ketamine (95 mg) and xylazine (5 mg per kg body weight, respectively) and cardiac blood was collected prior to transcardial perfusion with ice-cold saline. Dissected ovaries were fixed in 10% buffered formalin and processed for paraffin embedding. Serum was separated from whole blood by centrifuge (10 min, 2000 rcf), transferred to a new 1.5 mL tube and stored at −80 °C. All animal care and procedures were conducted with approved institutional animal care protocols and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drug preparation and administration

Anastrozole powder (45 mg, AstraZeneca, UK) was mixed with red food coloring (40 μL, McCormick; Sparks, MD) and propylene glycol (1 mL, Fisher Scientific; Pittsburg, PA) on a glass slide forming a slurry, which was then transferred to a heated bag of no-sugar-added banana-flavored, hydration gel consisting of purified water, sucralose, fruit and natural flavoring, hydrocolloids, potassium sorbate, sodium benzoate, and phosphoric acid (8 oz LabGel; ~236 mL; ClearH_2O; Portland, ME). Pilot data demonstrated that animals