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Use of a Low-calorie Flavored Gel to Facilitate Oral Self-administration of Analgesics in Mice

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The goals of this study were to determine whether mice would adapt to a low-calorie flavored water gel as their sole source of hydration and whether the addition of acetaminophen, tramadol, meloxicam, or buprenorphine to the gel would affect their intake. Water and gel intakes were measured during a 4-phase study, each of which lasted 1 wk: phase 1, standard water bottle only; phase 2, standard water bottle and a separate tube containing water gel; phase 3, water gel only; and phase 4, water gel containing an analgesic drug. Water consumption, corrected for body mass, was not different between male and female mice when water was available (phases 1 and 2). However, the total consumption of water and water gel was higher for females than males during phase 2, and female mice consumed more gel than males during phase 3. When male and female data were combined, total corrected water intake was not different among the first 3 phases of the study. Gel intake did not change significantly after the addition of acetaminophen, meloxicam, buprenorphine or tramadol as compared with untreated water gel. These data suggest that drugs presented in the low-calorie flavored water gel may be a viable alternative to injection or gavage for the administration of analgesic drugs.

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Introduction

Safe and efficacious administration of analgesia to research rodents, especially mice, is an aspect of laboratory animal medicine and management that has scope for refinement. Analgesia regimens often involve the injection of analgesic drugs via intraperitoneal, subcutaneous, or intramuscular routes.[10,24] Not only does the injection itself lessen wellbeing in terms of pain, but the associated handling and restraint also can cause an avoidable stress response that may negatively affect the animal welfare and both behavior and physiology.[3,11] Nevertheless, the ethical acceptability of using animals in research requires the use of appropriate analgesia when warranted.

The use of oral analgesic therapies could mitigate these issues and avoid the need for repeated injections of analgesic drugs. An issue that frequently arises with oral administration of drugs in all species, including mice, is taste. Some drugs are extremely bitter and therefore unpalatable.[32] Therefore taste is a major limitation to animal self-administration of drugs by ingestion. While oral self-administration avoids stress and pain associated with injection or gavage of drugs, it relies on the animals willingly and consistently consuming the substance containing the drug. Oral self-administration can be used as an alternative to injections if drug administration is required for more than a few days if the gastrointestinal bioavailability of the drug is known.

MediGel Sucralose (Clear H_2O, Westbrook, ME) is a flavored water gel designed to facilitate oral administration of medication to research animals.[5] It contains 99% purified water and so is virtually equivalent in weight to water. To improve palatability, this water gel contains sucralose as a sweetener. This product was designed specifically for research animals and is marketed as a suitable sole source of water and as a mask for the flavor of medications.

The aims of this study were to determine whether mice would adapt to flavored water gel as their sole source of hydration and whether the addition of acetaminophen, tramadol, meloxicam, or buprenorphine would affect the intake of the gel.

Materials and Methods

This study was approved by the Animal Ethics Committee of University of Western Australia (WA) (approval number: RA/3/100/1735) and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (2013)[37] and Guidelines to promote the wellbeing of animals used for scientific purposes (2008).[36]

Animals. Arc:Arc(S) mice (an outbred albino stock; *Mus musculus*; 20 males and 20 nonpregnant females; age. 6 wk) were acquired from the Animal Resources Centre (Murdoch, Western Australia, Australia). These mice had not been genetically modified. The supplying facility was free from the following agents: mouse hepatitis virus, minute virus of mice, mouse parvovirus, murine rotavirus, mouse norovirus, Theiler murine encephalomyelitis virus, pneumonia virus of mice, murine cytomegalovirus, Sendai virus, mouse adenovirus type 1 and 2, lymphocytic choriomeningitis virus, Hantaan (Korean hemorrhagic fever) virus, ectromelia (mousepox) virus, reovirus, polyoma virus, K virus, lactate dehydrogenase elevating virus, mouse thymic virus, cilia-associated respiratory bacillus, *Clostridium piliforme*, *Mycoplasma pulmonis*, *Helicobacter* spp., *Streptococcus pneumoniae*, *Pasteurella pneumotropica*, *Salmonella* spp., *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Streptobacillus moniliformis*, *Pneumocystis murina*, and endo- and ectoparasites.

Housing. The mice were housed in a Physical Containment Level 2 zone within an AAALAC-accredited animal facility. Mice were housed in same-sex pairs in IVC (cage floor area, 19.5 × 28 cm; Maxi-Miser Positive Individually Ventilated System, Thoren Caging Systems, Hazleton, PA) that supplied

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HEPA-filtered air. Bedding material was course aspen chips (ABEDD SIA; Kalnciems, Latvia). Environmental enrichment included aspen gnawing blocks (Tapvei, Paekna, Estonia), paper towels (Kleenex, Tantanoola, South Australia, Australia), facial tissues (Kleenex), and cotton squares (Nestlets, Ancare, Bellmore, NY). Lighting intensity ranged from 150 to 950 lx on a schedule of 12 h white light, 2 h red light, and 10 h off automated via a programmable lighting system (Controlsoft, Cirencester, Gloucestershire, United Kingdom). Room temperature was maintained between 18 °C and 24 °C, and the ambient building humidity ranged from 30% to 70%. All mice were provided with ad libitum access to a commercially supplied diet (Meat Free Rat and Mouse Diet, Specialty Feeds; Glen Forrest, Western Australia, Australia) that was steam sterilized. Mice were transferred to new cages with fresh bedding and enrichment items every 2 wk.

Data collection. The mice were introduced into the facility 6 d before beginning the 5-wk experiment. The 40 mice were randomly allocated to 20 cages in same-sex pairs (20 males in 10 cages and 20 females in 10 cages). Four additional cages were set up without mice and contained the same source of water (water bottles or MediGel Sucralose) as the animal cages during the different phases of the study. These cages were used to measure losses caused by handling, leakage, and evaporation. Cages were allocated to each treatment in sequence, that is: cage 1, acetaminophen; cage 2, tramadol; cage 3, meloxicam; and cage 4, buprenorphine. This order was repeated for the remaining 16 cages. Mice were weighed twice weekly. Water and MediGel Sucralosewater gel were weighed on Mondays, Tuesdays, Wednesdays, Thursdays, and Fridays, as well as on Saturday and Sunday when the drugs were added to the gel. Animal wellbeing was evaluated at least 3 times each week by observation of activity, body posture, gait, social behavior, coat quality and skin condition, body condition, facial expression, and hydration. Each parameter was scored as 0 (normal), 1 (slightly abnormal), or 2 (moderately abnormal). These scores were summed, and planned interventions were used when the score was greater than 0; a score of 5 required immediate euthanasia. If a mouse lost more than 10% of its body weight during a particular phase of the study, monitoring was performed daily. If 15% was lost, the mouse was removed from the study.

Mice were transitioned from water to medicated water gel over 4 phases. Each phase lasted for 7 d (Figure 1).

Phase 1: measurement of water intake). All mice had ad libitum access to acidified drinking water (HCl; pH 2.5 to 3) in 550-mL bottles suspended in a wire rack from the top of the cage. The bottles were weighed daily from Monday through Friday. During the fortnightly cage change, care was taken to disturb water bottles as little as possible to minimize water loss from handling. The amount of water that was lost when the bottle was inverted (for placement in the wire rack) was recorded for each bottle.

Phase 2: measurement of water and gel intake. Flavored water gel (MediGel Sucralose, ClearH_2O, Westbrook, ME) was purchased from an Australian distributor; neither the manufacturer or distributor funded the study in any way. The gel was provided in a hard plastic tube with two 4 × 4-mm holes drilled into the side at the base to allow the mice access to the gel (Figure 2). The tubes were hung from the rack at the top of the cage with galvanized steel wire (Figure 3). Each cage had one tube containing 20 to 30 g of the gel. Each tube was weighed daily from Monday through Friday. Each morning, the tubes were gently shaken and the holes at the bottom were cleared of any congealed gel that might prevent access for the mice.

All cages contained a water bottle and a tube of water gel in its custom-made apparatus. The gel was mixed with green food coloring (Rainbow Food Colors, Queen Fine Foods, Alderley, Queensland, Australia) before being given to the mice. The coloring, while serving no experimental purpose during this phase, was included from the first introduction of the gel to accustom the mice to the gel and so prevent neophobic avoidance when the colored drug mixtures were added to the gel in phase 4. Water bottles and gel tubes were weighed daily from Monday to Friday.

Phase 3: measurement of gel intake. The water bottles were removed, leaving the gel as the sole source of hydration for the mice. The gel tubes were weighed daily from Monday through Friday

Phase 4: measurement of drug-containing gel. Various drugs were mixed with the gel. To allow visual assessment of homogenous distribution of drug in the gel, each drug was mixed with green food coloring prior to combining it with the gel. The desired final concentration of each drug in the water gel was calculated. Acetaminophen (100 mg/mL, Panadol Children 1 Month to 1 Year Baby Drops, GlaxoSmithKline, Ermington, New South Wales, Australia) was administered at 1.125 mg/g gel. Tramadol (100 mg/mL, Tramal Oral Drops, Seqirus, Parkville,

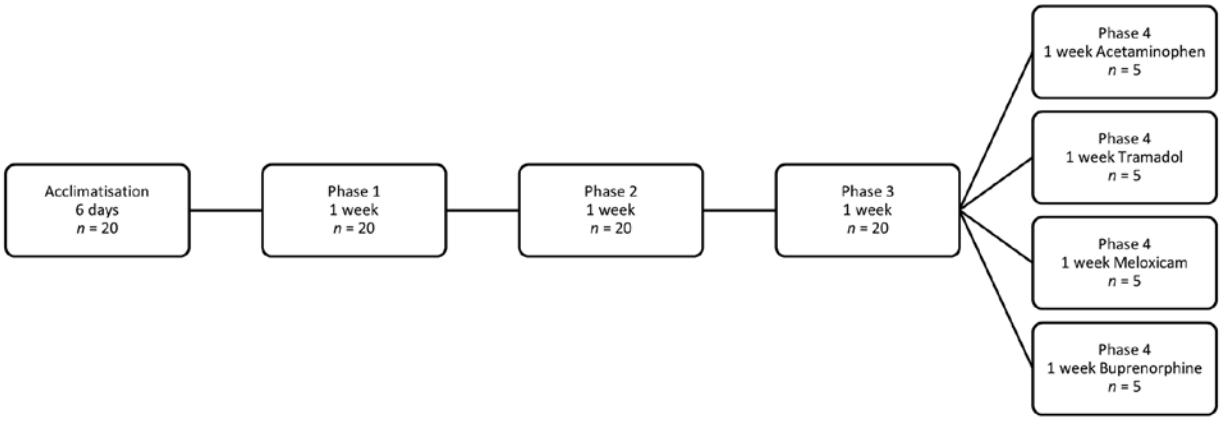

Figure 1. Overview of the project timeline. *n*, number of cages (cage 1 had one mouse; data from cages 2A and 2B, each with one mouse, were summed into a single cage; and all other cages had 2 mice each).

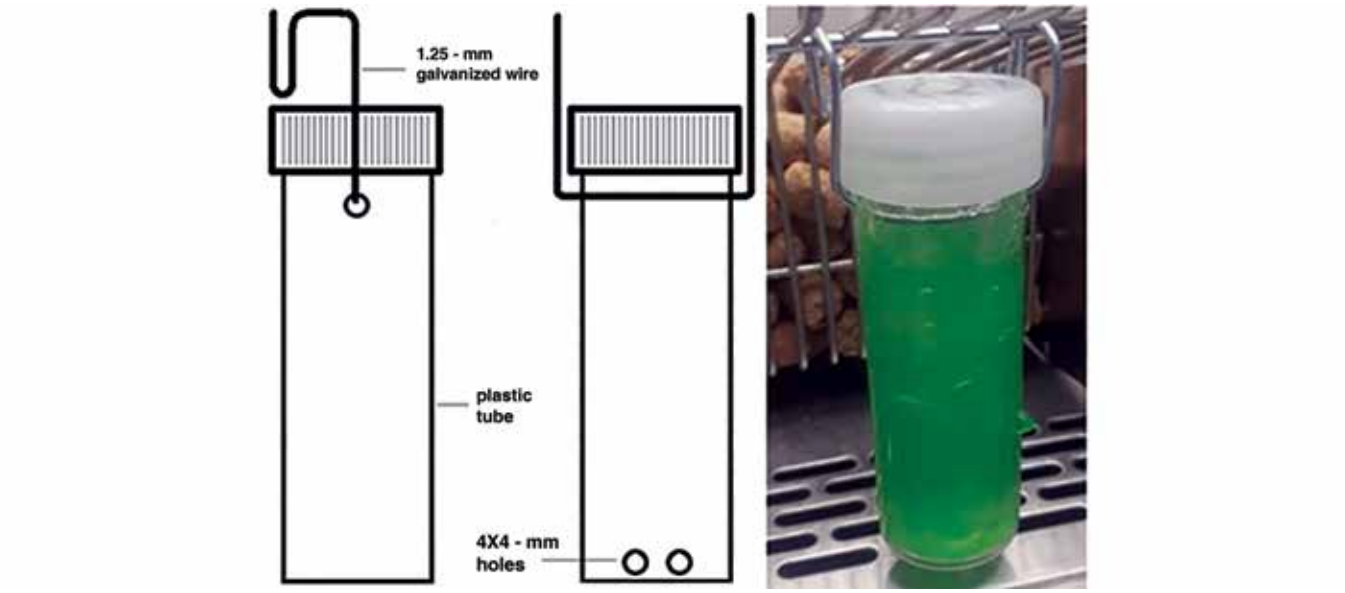

Figure 2. Gel tube apparatus, side view (left), front view (middle), and back view (right) of the tube in use.

Figure 3. Cage rack configuration. Placement of water bottle (top) and the custom-made tube apparatus (bottom).

Victoria, Australia) was administered at 0.1125 mg/g. Meloxicam (1.5 mg/mL, Apex Meloxicam Oral Suspension for Dogs, Dechra Veterinary Products, Somersby, New South Wales, Australia) was administered at 0.017 mg/g. Buprenorphine (0.2 mg, Temgesic Sublingual Tablets, Indivior, Macquarie Park, New South Wales, Australia) was administered at 0.0005 mg/g.

Preparation of medicated gel. The concentration of each drug in gel for phase 4 of the experiment was calculated based on the weight of mice and the average gel intake during Phase 3, such that a mouse of average weight and gel intake of either sex would consume a therapeutic dose. The concentration of the medicated gel was the same in the male and female cages to maintain the same palatability. Due to uncertainty regarding whether the addition of the drugs would affect gel consumption, conservative concentrations were chosen to minimize the risk that smaller mice would consume more than recommended and thus experience adverse side effects. Drugs were chosen based on common usage for rodents in research settings and documented efficacy when administered orally in mice. The measured intake of gel during phase 3 was used to determine the final concentration of each drug. Each of the four drug-gel mixtures were prepared in 200 g batches to ensure accuracy of the drug concentrations. The batches were refrigerated for 1 to 2 d and were then discarded and new batches prepared.

Fate of the mice at the end of the study. At the end of the study, all mice were euthanized via CO_2 inhalation overdose. Mice were placed in a 10-L chamber and slowly introduced to 100% CO_2 at an initial rate of 3 L/min, which was increased to 5 L/min after the mice were confirmed to be unconscious by lack of righting reflex.

Statistical analysis. Independent *t* tests were used to compare the effect of sex on water and gel intake. Paired *t* tests were used to compare the intake of water (or gel or both) between the first 3 phases of the study and to assess the effect of adding analgesics to the gel (phase 3 compared with phase 4). Data are expressed as daily intake (mean ± SE) in milliliters per gram of body weight. Samples sizes (n) refer to the number of mouse cages in each statistical comparison. The inclusion of 20 cages in this study, a clinically meaningful reduction in water-equivalent intake of 10%, and an assumed standard deviation of 0.027 mL/g daily represented a power of 74%. Stata (version 16.1, StataCorp, College Station, TX) was used for all analyses. A *P* value of less than 0.05 was defined as statistically significant.

Results

One of the male mice from cage 1 died of unknown causes before the start of data collection, but this cage was still counted as one of the 20 cages. Two male mice allocated to cage 2 were separated on day 2 of the study due to fighting (boxes 2A and 2B). The measurements from these 2 boxes were summed and then included as a single cage. Otherwise, animal

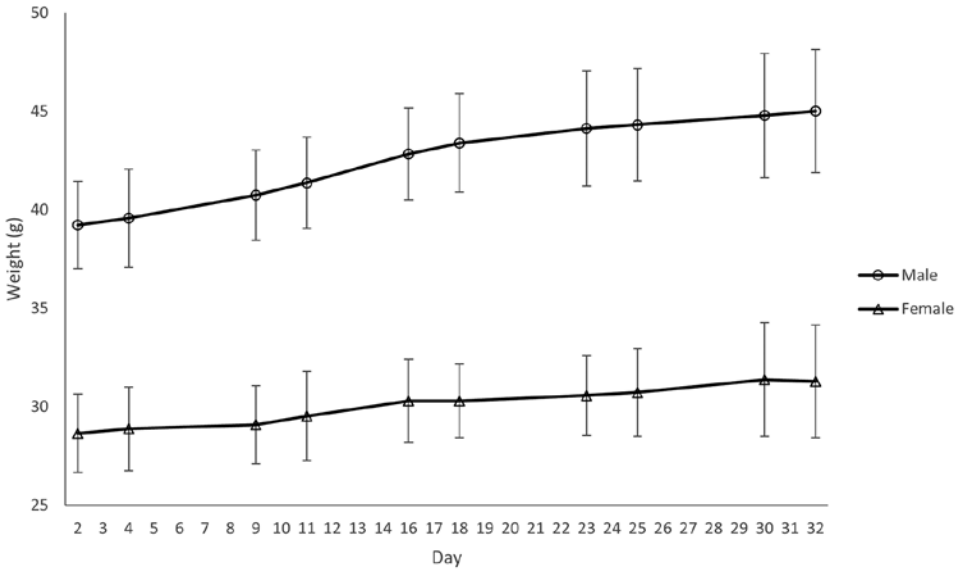

Figure 4. Body weight (g; mean ± SE [bars]) of male and female mice at 2- to 5-d intervals. Days 1–7, phase 1; days 8–14, phase 2; days 16–21, phase 2; days 22–28, phase 3; and days 28–32, phase 4.

Table 1. Comparison of corrected consumption of water or gel or both between male and female mice during phases 1 through 3

	Daily consumption (mL/ g) of water ± flavored water gel according to sex	*P*
Phase 1: Standard water bottle	Water	0.654
	Males, 0.144 ± 0.006	
	Females, 0.148 ± 0.006	
Phase 2: Standard water bottle and a separate tube containing water gel	Water	0.230
	Males 0.071 ± 0.002 water	
	Females 0.084 ± 0.009 water	
	Gel	0.085
	Males 0.050 ± 0.008 gel	
	Females 0.083 ± 0.016 gel	
	Water + gel	0.004[a]
	Males, 0.121 ± 0.007	
	Females, 0.166 ± 0.011	
Phase 3: Water gel in a tube	Gel	0.036[a]
	Males, 0.139 ± 0.009	
	Females, 0.163 ± 0.006	

Each phase comprised 20 cages housing same-sex pairs.
[a]Consumption differed significantly ($P < 0.05$) between sexes.

wellbeing was acceptable during the study, with no adverse reactions to the gel or drugs, and a planned intervention was not needed.

In phase 2, water gel initially was placed on the floor of each cage in the plastic cup in which it was supplied. Within 24 h of introduction of the gel, we noted that mice filled the cups with bedding and feces, thus precluding accurate measurement of gel intake. Therefore, we fabricated tubes to use as the vessels for gel administration (Figure 2). The data for this first week were not included in the analysis, because we could not collect a complete data set; phase 2 data collection began the following week.

Throughout phases 2 through 4, many of the mice chewed around the lids of the gel tubes. Each new, unchewed lid was approximately 2 g, and the removal of this small amount of material was deemed negligible to the overall measurements. Each empty tube weighed approximately 13 g, including the lid and the wire.

To convert the weights of water and gel to a volume, we assumed that because the gel was 99% purified water,[5] 1 g of water was equivalent to 1 mL; 1 mL of gel was weighed and confirmed to weigh 1 g.

At the end of phase 3, the male mice weighed (mean ± SE) was 44 ± 3 g and female mice weighed 31 ± 2 g. The mean daily gel intake was 7.4 ± 1.4 g for male mice and 5.6 ± 0.7 g for female mice. Mice gained weight throughout the study (Figure 4).

The equivalent water consumption divided by the animals' body weights (hereafter, 'corrected' values) was analyzed. This approach reflects the industry standard in similar veterinary studies.[2,41] The data showed no correlation between the weight of the mice from the same cage and consumption by each sex. However, male weighed more and consumed more than female mice. Therefore, before analysis, we normalized weekly consumption by dividing it by the average weight for each sex rather than by the weight for each pair.

When water was available (phases 1 and 2), corrected water consumption did not differ between males and females (phase 1, $P = 0.654$; phase 2, $P = 0.230$). However, combined consumption of water and gel during phase 2 was higher for female than for male mice ($P = 0.004$), and female mice consumed more gel than males in phase 3 ($P = 0.036$; Table 1).

When data from male and female mice were combined, total corrected water intake did not differ between the first 3 phases of the study (Table 2).

With male and female data combined and compared with that of untreated gel, corrected gel intake did not differ after the addition of acetaminophen ($P = 0.218$), meloxicam ($P = 0.138$), buprenorphine ($P = 0.248$; Table 3 and Figure 5) or tramadol ($P = 0.082$). The intakes of medicated gel during phase 4 met that the mean daily recommended doses of acetaminophen and tramadol but meloxicam or buprenorphine did not (Table 4).

Discussion

The primary aims of this study were to determine whether mice would adapt to a flavored water gel as their sole source of hydration and maintain this intake after therapeutic doses of

acetaminophen, tramadol, meloxicam, or buprenorphine were added to the gel. Corrected total water intake did not differ between the first 3 phases of the study, so the mice successfully adapted to the water gel as their sole source of hydration. Furthermore, the addition of an analgesic drug did not change significantly change the corrected water intake. However, the results of this study should be viewed conservatively due to its small size.

The gel introduced in phase 2 was mixed with green food coloring, and later in Phase 4, the drugs were mixed with the food coloring before being given to the mice. The purpose of phase 2 was to allow the mice time to familiarize themselves with the gel as a source of hydration before the water bottles were removed, given that mice are notoriously neophobic. The color green was used based on the assumption that mice have dichromatic vision[6,15] and would not have an aversion to the color green because they cannot perceive it.

Table 2. Combined data from male and female mice for total corrected daily consumption (mL/g; mean ± SE) during phases 1 through 3

	Corrected daily consumption	*P*
Phase 1: Standard water bottle	0.146 ± 0.004	
Phase 2: Standard water bottle and a separate tube containing flavored water gel	0.144 ± 0.008	0.690 (phase 1 compared with phase 2)
Phase 3: Flavored water gel in a tube	0.151 ± 0.006	0.311 (phase 1 compared with phase 3) 0.215 (phase 2 compared with phase 3)

Each phase comprised 20 cages housing same-sex pairs.

Table 3. Daily consumption (ml/g; mean ± SE) of untreated or medicated water gel

	Daily intake of untreated water gel in phase 3	Daily intake of medicated water gel in phase 4	*P*
Acetaminophen	0.144 ± 0.014	0.155 ± 0.020	0.218
Tramadol	0.166 ± 0.010	0.133 ± 0.005	0.082
Meloxicam	0.158 ± 0.012	0.142 ± 0.015	0.138
Buprenorphine	0.135 ± 0009	0.152 ± 0.021	0.248

Each drug involved 5 cages, each housing one same-sex pair of mice.

After correction for mean body weight, the daily intake of water did not differ ($P = 0.654$) between sexes. The mean daily water intake that we observed—0.146 mL/g—is comparable to values found in the literature, although findings vary between sources. For example, the British Small Animal Veterinary Association[23] states that the average daily water consumption for adult mice is 15 mL per100 g body weight (0.15 mL/g), where another source[2] reported that the average water intake across 28 different mouse strains was 7.7 ± 0.3 mL per 30 g body weight (0.26 mL/g).

The flavored water gel that we evaluated (MediGel Sucralose, ClearH_2O) is advertised as able to provide the sole hydration source. The results we obtained here support this claim, given that the intake of water during phase 1 (0.146 mL/g daily) was comparable to the intake of gel during phase 3 (0.151 mL/g daily, $P = 0.311$).

Few current studies focus on the palatability of medications in veterinary species, but the human literature contains an abundance of reports. The administration of and adherence to prescribed medications for children has issues similar to those of veterinary species. However, animals generally do not experience the same taste sensation or range as humans. For example, cats (*Felis catus*) cannot detect sweetness,[27] and carnivorous mammals have few taste buds compared with herbivorous and omnivorous species.[18,26] Even closely related species like mice (*Mus musculus*) and rats (*Rattus norvegicus*) differ in their taste detection.[29]

The drugs used in this study were chosen as analgesic drugs that are commonly used in veterinary practice and laboratory animal medicine. They have varying degrees of palatability, and we expected this feature to have some effect on the consumption of the medicated gel. Tramadol is reported to have a very bitter taste to various species,[30,35,38] and buprenorphine reportedly is bitter to rats[16,30] whereas meloxicam is generally regarded as having acceptable palatability.[12,25]

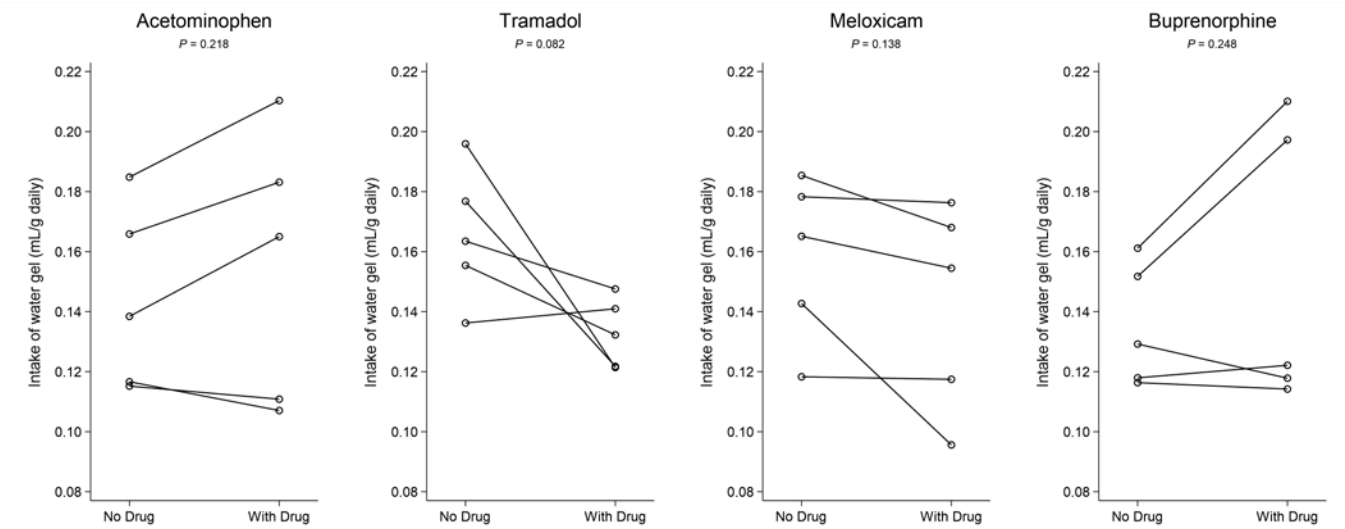

Figure 5. Corrected daily consumption of medicated water gel. Lines connect data points from the same mouse cage. The *P* values refer to the null hypothesis that the intake of water gel did not differ between before and after the addition of drug.

Table 4. Daily intake (mg/kg; mean ± SE) of drug in medicated water gel compared with recommended doses

	Suggested daily oral dose (mg/kg)	Daily intake (mg/kg) in phase 4
Acetaminophen	110–305 (reference 4)	174.375 ± 22.500
Tramadol	1.25–100 (references 28 and 42)	14.963 ± 0.563
Meloxicam	5 (reference 8)	2.414 ± 0.255
Buprenorphine	0.4 (reference 22)	0.076 ± 0.011

The acceptability of drug preparations is affected by excipients such as artificial sweeteners, The acetaminophen used in this study was a children's preparation that already contained an artificial sweetener (saccharin sodium). Acetaminophen is known to be generally unpalatable regardless of the preparation;[13,40] however the additional sweetener in the preparation could have enhanced the palatability of the acetaminophen gel and resulted in greater consumption than if no additional sweetener was present. Postoperative water intake is higher in mice and rats if the water contains therapeutic concentrations of an acetaminophen preparation registered for children.[9,33] It is not only the presence of a sweetener that can alter the intake of medicated water, but the effect of the drug itself should also be considered. It is also possible that the analgesic effects of the drugs consumed influenced the intake of medicated water.[1,10,11] For the analgesic tramadol, the dose administered influences the intake of both water and food. Mice given high doses of tramadol in drinking water had significantly lower food and water intakes and lost weight, compared with mice in the low-dose group, yet developed no additional analgesia.[19] That study also showed that buprenorphine and low-dose tramadol in the drinking water were comparable with one another in their effects on food consumption and body weight. Several studies have suggested that buprenorphine ingested voluntarily via drinking water may be a useful way to provide adequate analgesia to mice and rats undergoing painful stimuli, although results were better when treated drinking water was used in conjunction with injectable administration.[17,19,20,39]

Several studies have investigated the use of highly palatable sweet nut pastes, such as Nutella (Ferrero, Alba, Italy) and Medigel Hazelnut (ClearH_2O) as an aid in oral self-administration of various drugs, with promising results.[1,7,16,21,22] However, sweet nut pastes contain high quantities of sugar and fats that may be undesirable in some studies. Using water with highly palatable additives, such as sweet nut pastes, or pre-sweetened drug preparations (e.g., children's acetaminophen) creates the risk of drug overdose. Studies using Nutella generally provide a measured amount of the paste–drug mixture that will provide the desired dose while remaining palatable. Similarly, buprenorphine-medicated pellets are well accepted by mice and can be used to provide adequate analgesia.[34] The flavored water gel we used contains the artificial sweetener sucralose instead of sugar and so may present a viable alternative that avoids consumption of additional sugar or fats.

Our data indicate that the intake of flavored water gel is maintained after the addition of acetaminophen, meloxicam, buprenorphine and tramadol. All groups remained clinically well and sufficiently hydrated.

Several limitations of this study should be considered when interpreting the results. The study focused solely on the effects of 4 analgesic drugs on the intake of a low-calorie flavored water gel. Analgesic efficacy of the drugs was not evaluated, although the observed daily drug intakes can still be compared with published doses (Table 4). This comparison showed that the observed daily intake of meloxicam and buprenorphine was below published ranges. One group[14] used the same gel we evaluated to show that mice will consume buprenorphine at concentrations of 5 and 15 µg/mL achieving serum concentrations in a range considered to be analgesic.[22] Future studies should evaluate intake of water gel with higher concentrations of meloxicam. The safety of the drugs in the present study was evaluated only through observational assessment of behavior and activity. In addition, study numbers were relatively small, resulting in relatively low power to detect clinically significant differences between phases of the study.

This study has a number of potential future applications. One possibility is the provision of preemptive analgesia. Although we did not compare the rates at which mice acclimated to the water gel, our results provide a starting point for transitioning mice to the product. If mice require but do not receive acclimation to water gel, they might not consume therapeutic doses of analgesia. Once mice successfully transitioned to water gel, the provision of analgesics before painful procedures could minimize the stress associated with repeated handling and restraint as well as the pain of injectable analgesia. Our study does not provide any information regarding the intake of MediGel Sucralose after a painful procedure. Naturally, if the intake of water gel decreased postoperatively, then analgesic intake would decrease also.

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