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FULL ARTICLE

Telmisartan detection by UV spectrophotometry in mice drinking water

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Abstract

Animal models are often used to test the safety and efficacy of drugs in cell culture and body systems. Several researchers deliver drugs to rodents in drinking water, although it has some limitations. For instance, drug stability, water consumption, and body mass fluctuation may change drug dose. Thus, we investigated telmisartan (TEL) stability in mice drinking water by UV spectrophotometry, and if water intake and body mass were fluctuated then it changes the predicted drug dose. The results showed that UV spectrophotometry could detect TEL at the wavelength of 300 nm, and the concentration curve was set between 1.25 and 60 μg/mL. Also, it remained stable in mice drinking water for 7 days at the predicted concentration. Mice gained weight after 8 weeks on a high-fat high-sucrose diet, and it was reduced by TEL 5 mg/kg/day after 3 weeks. Although water intake remained stable, not adjusting the TEL concentration weekly by body mass would lead to higher consumption of TEL by mice. In conclusion, we demonstrated that body mass and water intake fluctuations significantly change the amount of drug that the animal receives, which would add bias to the experiment. TEL remains stable for at least 7 days in wrapped mice water bottles in the animal care facility, and UV spectrophotometry proved to be a simple and low-cost method to detect TEL in mice drinking water.

KEYWORDS
C57Bl/6 mice, drinking water, drug administration, telmisartan, UV spectrophotometry

1 | INTRODUCTION

Animal models are widely used to understand the onset and development of diseases. They are also used to test the safety and efficacy of drugs, as well as the pathophysiology and molecular mechanisms underlying drug effect on body systems. Drugs can be delivered to rodents in many ways, such as diluted in the drinking water and chow, into the stomach by oral gavage, and intraperitoneally. Each route has its advantages and disadvantages, and the most commonly used routes are oral gavage[1,2] and drinking water.[3–5] Drinking water is the easiest route for drug delivery since it does not stress the animal, it does not require specific skills or training, and it has low cost and low risk for the animal compared to other methods.

On the other hand, rodents might not receive the planned drug dose because water intake varies along with the experiment. Also, in obesity studies where rodents gain or lose weight, drug dosage is affected. Another concern is drug stability since some substances lost their activity when exposed to light. Thus, the researcher must assure that the animal ingested the planned dose and that the drug solution is stable.

Telmisartan (TEL) is an antihypertensive drug approved by the US Food and Drug Administration since 1998.[6] It is an angiotensin II type 1 (AT1) receptor blocker, and it is chemically described as 4′- [(1,4′dimethyl-2′-propyl [2,6′-bi-1*H*-benzimidazol]-1′yl) methyl]-[1,1′-biphenyl]-2-carboxylic acid.[7,8] TEL reduces blood pressure and prevents cardiovascular morbidity and mortality since it reduces left ventricular hypertrophy, arterial stiffness, and atrial fibrillation.[8,9] TEL also exerts pleiotropic effects on glucose metabolism and insulin sensibility due to its action as a partial peroxisome proliferator-activated receptor γ (PPARγ) agonist.[1,8] Many studies have been conducted in rodent models of hypertension,[10,11] diabetes,[12,13] and obesity[14,15] to investigate how TEL acts on blood pressure and glucose metabolism. TEL-induced body weight loss is dose and time dependent; thus, it is essential to ensure that the animals ingested the planned dose. Light sensibility by TEL is another concern.

There are many methods to analyze the TEL concentration in solution, such as ultraviolet (UV) spectrophotometry, immunoassay, liquid chromatography-mass spectrometry (LC-MS),[16] high-performance liquid chromatography (HPLC),[17,18] ultra-performance liquid chromatography,[19] and also polarography and visible spectrophotometry.[16] Except for spectrophotometry, these techniques are expensive, require high-cost equipment, demand expert training, and use solvents that can be harmful to health if incorrectly used.[16] Thus, the British Pharmacopoeia and the Indian Pharmacopoeia recommend UV spectrophotometry for TEL analysis.[19]

Therefore, our goal was to exploit how to assure the planned drug dosage when the drug is administered to mice that are subjected to water intake and body weight fluctuations throughout the experiment, using TEL as an example. Also, we evaluated if it is possible to measure the TEL concentration and stability in the drinking water offered to mice. To accomplish that, we used the method of UV spectrophotometry proposed by Chavhan et al to measure the TEL concentration in the mice drinking water.[20]

2 | MATERIALS AND METHODS

2.1 | Ethical aspects

The local ethics committee approved the handling and experimental protocols to Care and Use of Laboratory Animals (CEUA#647/15). The study was performed following the Animal Research Reporting In vivo Experiments ARRIVE guidelines and the Guideline for the Care and Use of Laboratory Animals (US NIH Publication no. 85-23. Revised 1996).[21]

2.2 | Animal husbandry and diet

Twenty female C57BL/6 mice at 2 months old were maintained in collective polycarbonate microisolators of 30 × 20 × 23/28 × 18 × 11 cm external/internal dimensions, with a wire bar lid that serves as food hopper and water bottle holder on ventilated racks (Scienlabor Industrial Equipment, SP, Brazil). Five mice were housed per cage. The housing conditions were 12 h light/dark cycles, 21 ± 2°C, 60 ± 10% humidity, and an air exhaustion cycle of 15 min/h. At 3 months old, mice were feed for 8 weeks with a purified AIN93M diet,[22] or a high-fat high-sucrose diet (HFHS) modified from the AIN93M diet (Pragsolucoes, Jau, Sao Paulo, Brazil) to induce weight gain. TEL 5 mg/kg/day was administered ad libitum to mice in their drinking water for 3 weeks until the 11th week of diet intake (Figure 1).

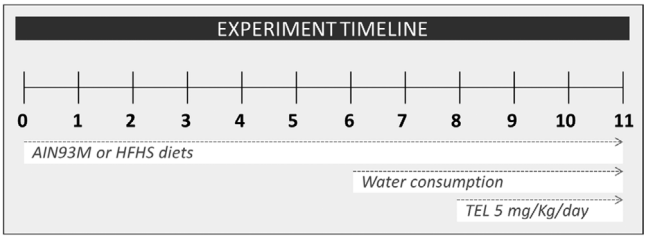

FIGURE 1 Experiment timeline (weeks). Mice were fed during 11 weeks with control AIN93M diet or a modified AIN93M diet rich in fat and sucrose (HFHS). Water intake was measured for 2 weeks before TEL administration, and then daily during TEL administration. TEL was offered for 3 weeks in the drinking water at 5 mg/kg/day. Abbreviations: HFHS, high-fat high-sucrose diet; TEL, telmisartan

2.3 | Instrumentation

The spectrophotometric measurements were carried out using an Epoch UV-visible light spectrophotometer (BioTek® Instruments, Vermont, USA). It consists of a photodiode detector with a xenon flash light source. Optical performance is a λ range of 200–999 nm, ±2 nm accuracy, ±0.2 nm repeatability, 5 nm bandpass, and 0.000-4.000 optical density (OD). Readings were performed in a 96-well microplate. An analytical scale (Shimadzu, AUW220D, Kyoto, Japan) was used for reagent weighing.

2.4 | Reagents

TEL 80 mg (Lot. 2680246, Ranbaxy Laboratories Limited, Mohali, Punjab, India) was bought from a local market. Milli-Q purified water was used for reagent dilution (Millipore, Massachusetts, USA).

2.5 | Solution preparation for TEL assay

The solution was prepared based on the method of Chavhan et al. They showed that TEL has better solubility in 0.1 N NaOH. Also, the TEL (Ranbaxy Laboratories Limited tablet) concentration claimed in the label matched with the estimated concentration of 100 mg/tablet by 99.15% after six determinations.[20] Thus, we used TEL tablets to prepare the standard and working solutions for the determination of TEL in drinking water.

Standard stock solution (1 000 µg/mL): The average weight of five tablets was determined. The powder equivalent to 50 mg of TEL was weighed and dissolved in 25 mL 0.1 N NaOH by sonication (570 s, 42 kHz, 60 W, Ultrasonic Cleaner CD-4800, Practical Systems, Florida, USA). Then, it was passed through a filter paper of 14 µm pore, and the volume was made up to 50 mL with 0.1 N NaOH to give a 1 000-µg/mL stock solution.

Working stock solution (100 µg/mL): 2.5 mL was withdrawn from the standard stock solution and diluted to 25 mL with 0.1 N NaOH to obtain a 100-µg/mL solution.

Selection of analytical wavelength: The absorbance of working solution and blank was sampled in the range of UV radiation from 200 to -400 nm at 5 nm intervals. TEL showed the maximum absorption at the wavelength (λ_{max}) 300 nm (Suppl. 1A).

Calibration curve (1.25-100 µg/mL): TEL working solution was diluted to obtain a concentration range of 1.25, 2.5, 5, 10, 20, 40, 60, 80, 100 µg/mL in Milli-Q purified water. In a 96-well microplate, 300 µL of each solution was read in the range of 200-400 nm at 5 nm intervals in triplicate, and the absorbance at 300 nm was used to calibrate the standard curve (Suppl. 1B). Calibration solutions showed a linear response with increasing concentration. A curve range of 1.25-60 µg/mL was chosen, based on the goodness of fit of the linear regression (R squared = 0.9989, Sy.x 0.01929) and Pearson correlation ($r = .9994$, $P < .0001$) (Suppl. 1C).

2.6 | TEL dilution in mice drinking water

Previous researches show that the drinking water is the better route for TEL administration to mice.[3,5,23] Due to space limitation, mice are often housed in collective cages. Therefore, water intake is evaluated per cage, and water consumption represents the average of five mice, not individual intake. Water consumption was measured daily from Monday to Friday throughout 2 weeks before TEL administration to estimate average water consumption. Daily water intake was used to determine the amount of TEL required to prepare the TEL drinking solution. We needed to assure that the volume of water ingested by each mouse in one day had the amount of TEL planned (5 mg/kg/day). Water intake was also monitored during the experiment since changes in water consumption would change TEL dosage.

Table 1 shows the amount of TEL powder (macerated tablet) required to be diluted in mice drinking water was determined. First, it was calculated how much TEL (drug itself) would be necessary daily for mice (110.0 µg/day/mice), based on average body weight of mice housed in the same collective cage (22.0 g). Second, the result was corrected based on the amount of pure TEL presented in the TEL powder (in this example, 660.0 µg/day/mice of TEL powder). Finally, to know the amount of TEL powder required for dilution in mice drinking water, it was necessary to know daily water intake (3.2 mL/day/mice, an average of five mice housed in the same cage) to assure that the planned TEL quantity (i.e., 666.0 µg of TEL powder) is found in 3.2 mL of water.

TEL was offered to mice in water bottles wrapped in aluminum foil to protect the solution from exposure to environmental light since TEL is light sensitive. TEL was provided ad libitum in 300 mL water bottles for 3 weeks to evaluate its impact on body mass. Body mass was assessed weekly. The amount of TEL diluted in mice drinking water was adjusted weekly based on the average body mass and water consumption of the previous week for each mice cage.

TABLE 1 Simulation showing how to calculate Telmisartan weight required to prepare mice drinking water

Telmisartan dose calculation[a]	
Body weight	22.0 g
Telmisartan dose	5.0 mg/kg/day
Telmisartan (tablet powder)	80 mg in 479.8 mg powder
Telmisartan (pure)	110.0 µg/day/mice
Telmisartan (powder)	660.0 µg/day/mice
Drinking water preparation[a]	
Water volume	300.0 mL/bottle
Water consumption	3.2 mL/day/mouse
Telmisartan required (powder)	61.85 mg

[a] Body weight and water consumption are illustrative to understand calculations, based on averaged data obtained during the experiment.

2.7 | TEL concentration and stability in mice drinking water

Since our concern about the TEL concentration and stability emerged after the experiment was finished, we designed an assay to simulate the conditions to which TEL solution was subjected during experimentation. Thus, four water bottles were filled with a TEL solution consisting of 22.15 mg of TEL tablet powder diluted in 100 mL of filtered water (~36.93 µg/mL of pure TEL).

These water bottles were wrapped in aluminum foil and left at animal's room facility for 1 week. One sample of each bottle was collected at 0, 1, 2, and 7 days after solution preparation to evaluate the TEL concentration and stability. In a 96-well microplate, 300 µL of filtered water (blank), TEL solution or TEL calibration curve solutions (1.25–60 µL/mL) were read at 300 nm in triplicate. The TEL concentration was calculated from the standard curve at 300 nm.

2.8 | Expected and observed TEL concentration in mice drinking water

To precisely offer the intended (expected) dose of TEL, it requires the knowledge of individual body mass and water intake. Since mice were housed in collective cages, we knew their body mass, but not water intake because the last was an average of water intake per cage. It might be a critical issue for drug delivery because some mice would consume more (or less) drug. Thus, we first evaluated if using the average water intake per cage would significantly change the intended (expected) TEL dosage by mouse. For this analysis, we calculated the

TABLE 2 Average water intake (mL/mouse) before and after Telmisartan administration to mice

	Control Tel		HFHS Tel		
Week	Cage 1	Cage 2	Cage 3	Cage 4	*P*
−2	3.3 ± 0.7 (20.6)	3.1 ± 0.5 (15.6)	3.9 ± 1.0 (23.7)	3.9 ± 1.0 (24.2)	.53
−1	3.2 ± 0.6 (19.1)	3.3 ± 0.4 (10.9)	3.8 ± 0.4 (10.1)	3.7 ± 0.4 (9.5)	.51
0	3.6 ± 0.7 (20.3)	3.8 ± 0.8 (21.1)	4.1 ± 1.2 (29.5)	4.1 ± 1.0 (25.2)	.92
1	4.0 ± 0.5 (12.9)	4.0 ± 0.5 (12.6)	4.0 ± 0.5 (12.1)	4.3 ± 0.4 (12.1)	.54
2	4.0 ± 0.4 (10.2)	4.5 ± 0.4 (8.5)	4.5 ± 0.7 (16.1)	4.7 ± 0.7 (15.7)	.26
3	4.9 ± 1.2 (23.7)	4.1 ± 0.4 (9.7)	4.1 ± 0.2 (5.8)	4.6 ± 0.4 (7.9)	.50

Five mice were allocated per cage.
Average water intake represents daily measurements from Monday to Friday (total of five readings), except for the last week, where cages 1 and 2 were read three days and cages 3 and 4 read 4 days.
Data are expressed as mean ± SD (C.V.).
Nonparametric one-way ANOVA Kruskal-Wallis and post hoc Dunn's multiple comparison tests were performed. The comparison was not made between weeks since it is expected that water intake presents time-dependent fluctuation.

expected TEL concentration using individual mouse body mass and the average water intake per cage, and the observed TEL concentration was calculated based on the average body mass and water intake per cage. Second, we investigated if the observed TEL concentration differed from the expected if the water intake and body mass were not recalculated weekly. For this analysis, the expected TEL concentration was also calculated using individual body mass and average water intake, whereas the observed TEL concentration was based on average water intake and body mass measured before TEL treatment, and it was not recalculated along the next 3 weeks.

2.9 | Statistical analysis

Data are expressed as mean ± SD and analyzed for normality and homoscedasticity of variances. Linear regression was used to generate the standard curve and to calculate the TEL concentration. One-way ANOVA of Kruskal-Wallis and post hoc Dunn's multiple comparisons were used to compare water consumption among cages. Water consumption and body mass were analyzed by repeated-measures ANOVA with post hoc Sidak's multiple comparison test and linear trend. Observed and expected TEL concentration was compared with the one-sample *t*-test, paired *t*-test, and Bland-Altman plot. A *P*-value < .05 was considered statistically significant (GraphPad® Prism software v. 8.0, La Jolla, CA, USA).

3 | RESULTS

3.1 | TEL detection by UV spectrophotometry and stability in mice drinking water

We confirmed that TEL is detectable by UV spectrophotometry. The working stock solution showed the maximum absorption at the wavelength (λ_{max}) of 300 nm (Suppl. 1A). This wavelength was used to determine the optical density of a series of TEL dilutions (Suppl. 1B) to establish the standard curve (Suppl. 1C). Once the standard curve was established, the TEL concentration in four water bottles containing a known concentration of TEL (37.0 μg/mL) was assessed, to evaluate if it would remain stable along 7 days (Suppl. 1D). The observed and expected TEL concentrations were similar, showing that TEL remains stable in the drinking water for at least 7 days.

3.2 | TEL did not change water intake and induced body weight loss

Water intake was assessed 2 weeks before TEL offering to mice (weeks −2, −1, and 0, Table 2) and during the 3 weeks of TEL intake, to evaluate if TEL changes water consumption (Table 2). Despite tight control of animal care environmental conditions, our experience shows that water intake usually displays small fluctuations both up and down over the days, despite the experimental design. For this reason, we compared the average water intake among cages on the same week, but not among different time points. Data show that water intake was similar among cages, showing that TEL does not interfere with it (Table 2). When calculated as mL/kg body weight, water intake was also similar among cages (data not shown). Eight weeks on HFHS diet lead to body mass gain, and 3 weeks of TEL administration reduced it (Figure 2A). In summary, water intake was slightly increased, and body mass decreased from weeks 0 to 3. For this reason, the TEL concentration in mice drinking water was recalculated and intentionally reduced; otherwise, mice would receive more TEL than the initial dose planned of 5 mg/kg/day.

3.3 | Observed and expected TEL concentration in mice drinking water

Figure 3 shows the expected (planned) and observed TEL concentration in the drinking water in two different situations. In the first scenario, the TEL concentration was adjusted every week based on mouse

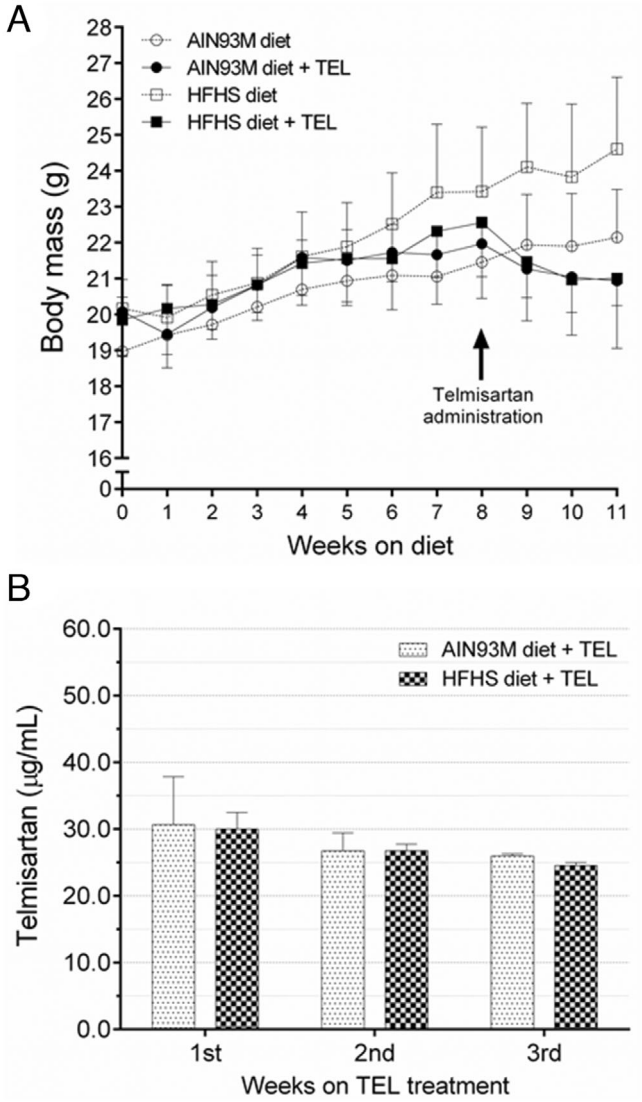

FIGURE 2 Body mass and TEL dose adjustment. (A) Body mass of mice fed for 11 weeks with control AIN93M diet or a modified AIN93M diet rich in fat and sucrose (HFHS). From 8th to 11th week, TEL was offered to mice in the drinking water. * indicates HFHS diet versus HFHS diet + TEL, $P < .05$, *t*-Student test. (B) Adjustment of TEL concentration in mice drinking water due to TEL-induced body weight loss. Statistical comparison was not performed since $n = 2$ cages/group (mice were housed in collective cages)

body mass (individual) and water intake (cage average), and, as a result, observed and expected TEL concentrations were similar (Figure 3A). In the second scenario (Figure 3B), we plotted the observed TEL concentration if we used body mass and water intake values obtained before the treatment started. We found that, if mice body mass and water intake fluctuation is not considered, there is a significant difference among observed and expected TEL concentration in the drinking water of the HFHS diet +TEL group.

In this second approach, the observed TEL concentration would be 13% higher ($P = .0059$) in the second week of experiment and 16% higher ($P = .002$) in the third week. Mice fed with the AIN93M diet showed no significant difference in observed and expected TEL concentrations, and it is likely because TEL did not lead to substantial changes in the body mass of this group. Comparing these two scenarios (adjusted vs nonadjusted TEL concentration) by the Bland-Altman plot, we noticed that not improving the TEL concentration weekly (Figure 3D) increased the bias and the difference between expected and observed TEL concentrations (Figure 3C).

4 | DISCUSSION

We investigated how to guarantee drug dosage when the compound is administered to mice in drinking water, knowing that body mass and water intake might change throughout the experiment and used TEL as an example. We saw that UV spectrophotometry could detect TEL at a wavelength of 300 nm, and it is stable in mice water bottles wrapped with aluminum foil for at least 7 days. If body mass and water intake changes throughout the experiment, it significantly changes the amount of drug that the animals will receive, adding a bias to the research.

The novelty of the present study is the proposal of an easy and nonexpensive method reproducible in every laboratory that works with basic science using rodents. Rodents are largely used to study drug effect on body systems in preclinical studies, but researchers do not use analytical methods as a routine to detect and monitor the drug offered to them, which can add bias to the research. The methodology proposed here might also serve as a model for detecting drugs other than TEL in mice drinking water. UV spectrophotometry is an easy and cheap method to detect the presence of drugs in rodents' drinking water, since other analytical techniques are expensive, require high-cost equipment, demand expert training, and use solvents that can be harmful to health if incorrectly used.

In the past decades, researchers have been developing and validating methods to quantify TEL in bulk and pharmaceutical dosage forms. Chavhan et al[20] used TEL diluted in 0.1 N NaOH and found the maximum absorption at 295 nm and linear function between 2 and 12 µg/mL. In our study, we followed the protocol described by Chavhan et al,[20] and we have found a maximum absorption at 300 nm. Regarding the TEL linear function range for detection, we decided for 1.25-60 µg/mL based on the goodness of curve fit of the linear regression. Our scale is wider compared to the previously published data, but they did not mention if they tested the TEL concentrations below or above the ranges reported on their works.

It is interesting to note that, in a review by Patel and Patel, it was discussed that TEL determination by the UV method could suffer interference due to other UV absorbing compounds. Therefore, the HPLC and HPTLC would be better methodsfor this kind of analysis. However, they are expensive and require elaborate procedures, which can make it difficult to implement.[24] Thereby, the UV spectrophotometry method is an easy, simple, and economical way to quantify TEL to evaluate its concentration in mice drinking water. We believe that the presence of other compounds might be a challenge for TEL determination in blood serum, for instance, but not when using water, NaOH, or methanol as

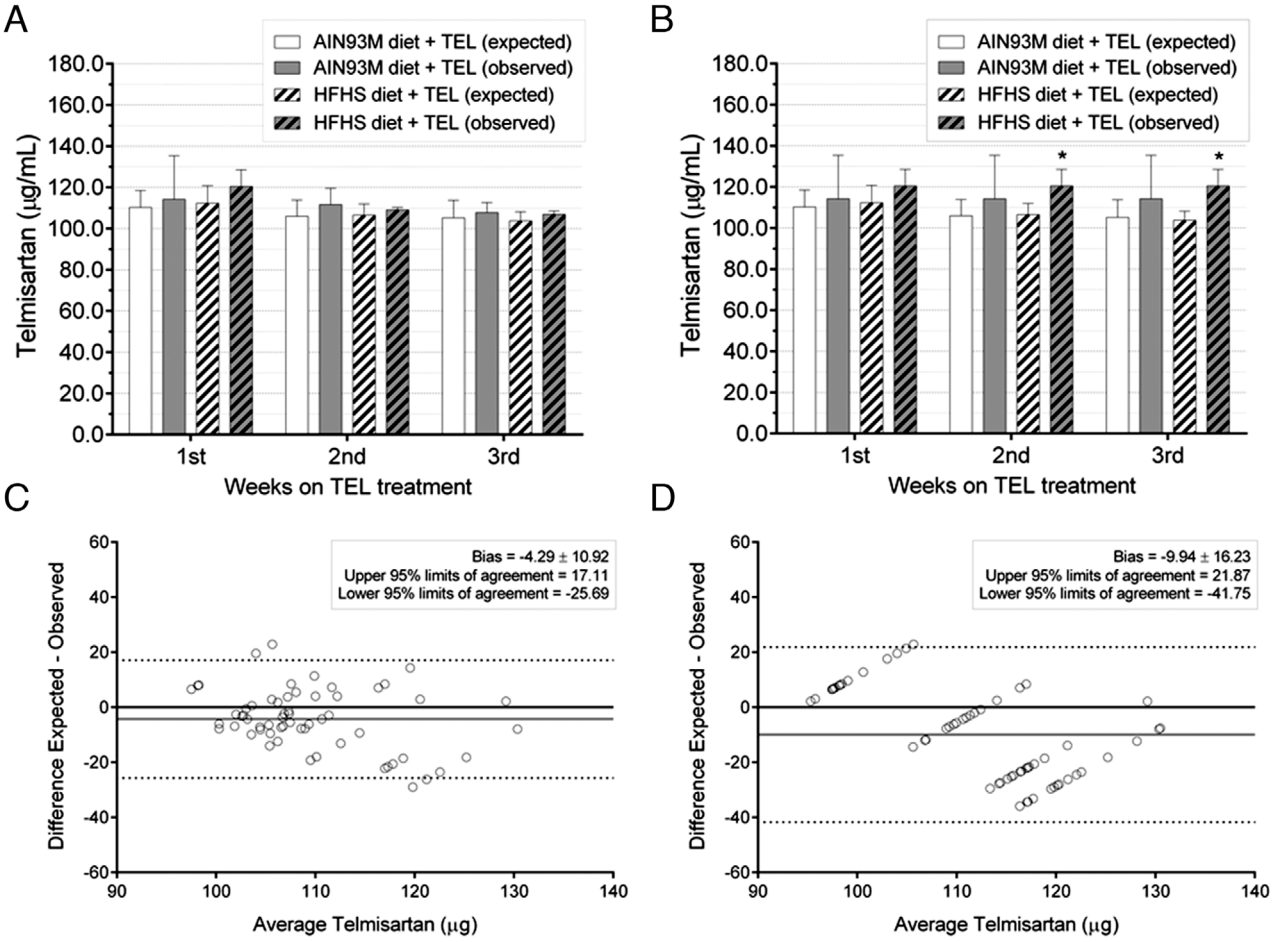

FIGURE 3 Expected versus observed TEL concentration in the drinking water offered to female C57BL/6 mice. (A) The average TEL concentration in mice drinking water was calculated twice weekly. (B) Average TEL concentration in mice drinking water, but it was based on average pretreatment water intake and body mass, thus it was not calculated twice weekly during the experiment. (C) and (D) Bland-Altman plot among the observed and expected TEL concentration in mice drinking water. In (C), the TEL concentration was recalculated twice weekly, but not in (D). When indicated, $P < .05$, comparing expected versus observed data (paired *t* test). Abbreviations: AIN93M, control diet; HFHS, high-fat high-sucrose diet; TEL, telmisartan

solvents. Finally, it is also important to highlight that TEL has no polymorphic behavior.[25]

The most common route for TEL administration is oral by ad libitum intake in the drinking water.[3,5,23] TEL dosage to C57BL/6 mice was chosen based on both human equivalent dose and after an extensive review of several studies. First, we considered the human dose of 40 mg/day, which is equivalent to approximately 8.2 mg/kg/day for mice.[26] The dose used in the literature ranges from 3 to 10 mg/kg/day. Based on this two information, we have chosen an intermediate dose of 5 mg/kg/day, which has been used in other studies with C57BL/6 mice and have shown biological effects on preventing body weight gain and body fat accumulation.[4,23]

In humans, TEL average terminal elimination half-life is between 20 and 24 h, contributing to the 24-h antihypertensive efficacy with once-daily dosing.[7–9,27] In mice, the terminal elimination half-life is 8–10 h,[7] which might be critical when it is provided in the drinking water since animals would regularly be exposed to the compound. It seems that rodents on a pelleted diet consume most of their water immediately before and after they eat food.[28] When housed under standard 12-h light/12-h dark cycle, mice consume most of their food during the dark, with short bouts during the light period.[29] To eliminate bias, an alternative would be to administer TEL twice or three times a day by oral gavage. However, the investigator must be trained on the technique, since unskilled personnel might stress and hurt the animal, by traumatic injury related to unappropriated restrain, by damaging the oral cavity, esophagus or trachea, or the solution might get access to the trachea and thus to the lungs, leading to animal death.

Zhang described a protocol for voluntary oral administration of drugs to mice, where the drug is offered in artificially sweetened and flavoured jelly and given to mice that have been trained to eat the jelly.[30] They report that mice need to be trained for 2-4 days, and they need to be individually housed to assure that every mouse eat the jelly and they do not fight for it, especially for male mice. The jelly is made of a mixture of Splenda® low caloric sweetener, water, gelatin, and flavoring essence imitation (strawberry of chocolate). This method is of interest since each jelly can be prepared with a different drug concentration

based on mouse body weight, which is not possible for drugs delivered in the drinking water. On the other hand, it is time-consuming, considering that one experimental group receiving the drug would have 8-10 mice that will be likely treated for at least one to several weeks.

Before providing drugs in the drinking water, the investigator must determine the average 24-h water consumption. Ad libitum water intake must be determined for the same strain, sex, age, and weight of the rodent strain that the investigator is willing to work.[31,32] In our experience, male C57BL/6 mice with 3-6 months old drink about 4-8 mL/day of water, and females about 3-5 mL/day. If they are submitted to a high-salt diet, water intake increases significantly, ranging from 8 to 20 mL/day for male and 7-14 for female C57BL/6 mice.[33,34] Therefore, water intake needs to be continuously monitored throughout the experiment to guarantee that the planned drug dose is indeed received. In the present work, we measured water consumption 2 weeks before the beginning of the experiment, but for the simplest determination, one may use three consecutive days.[31] Rodents must be housed in their usual cage to keep their routine. The water bottle that animals are already accustomed to is filled and weighted using a digital scale with 0.1 g accuracy. The bottle is weighed three consecutive days at the same time of the day to determine the average 24-h ad libitum water consumption; the measures are averaged and then divided by the number of animals in the cage.

We did not notice significant changes in water intake among the four mice cages. Corroborating with our data, TEL ad libitum to male Sprague-Dawley rats did not change water intake.[3] Depending on the drug used, it can have good palatability for mice, and thus, water consumption is increased. The opposite is also true, and therefore, the planned dose will vary. Additionally, it is essential to monitor food consumption. Fluctuations in food intake might change water intake, and also the compound itself might lead to an increase or decrease in food consumption. A reduction (or increase) in food consumption may change body mass and be a confounding factor to data interpretation regarding drug impact on body mass and body composition. Another example is the use of fructose solution to induce insulin resistance and hypertension in rodents.[35] In our experience, fructose offered to the rodent in drinking water increases water consumption. Therefore, care must be taken if the investigator is willing to administer a drug such as TEL into the drinking water when there is another factor influencing water intake (e.g., high-salt diet or fructose) because drug consumption will also change from what was planned.

As exposed, fluctuations in water intake and body mass impact drug consumption. Therefore, drug dose needs to be adjusted weekly. We showed that TEL decreased the body mass of mice from the HFHS diet + TEL group. If the TEL concentration were not regulated, mice would be exposed to a higher dose than expected, if the water intake did not change. In more extended study designs where mice display persistent weight loss, not adjusting TEL dosage would enhance its effects on body systems, adding a considerable bias to data interpretation. Not improving the TEL concentration did not impact mice from the AIN93M diet + TEL group, likely because they did not display a remarkable variation in body mass. Thus, if body mass did not change throughout drug intervention, drug adjustment might not be a critical issue.

Since TEL is offered ad libitum, it would be interesting to determine its blood concentration. In mice, peak plasma concentration (C_{max}) after one dose of 1 mg/kg of TEL is 162 ng/mL after oral administration,[7] but to date, we did not find a report for its plasma concentration when administered ad libitum to mice. Regarding methods to detect TEL, Salama reported the use of HPLC-UV in human plasma and was able to detect between 1 and 10 μg/mL of TEL.[36] An LC-MS method was also developed for TEL determination in human plasma,[37] but it requires a large blood sample, which is a limitation for mice studies. Overall, these techniques are expensive for laboratories that do not use them as routine. We attempted to determine TEL in mice serum using UV spectrophotometry, but unsuccessfully, since it required large blood samples to establish a concentration curve based on mice serum as a matrix, and also pure TEL instead of powder from tablet maceration.

5 | CONCLUSION

In conclusion, we demonstrated that body mass and water intake fluctuations throughout the experiment significantly change the amount of drug that the animal receives, and it would add a bias to the research. TEL remains stable for at least 7 days in wrapped water bottles in the animal care facility, and UV spectrophotometry proved to be a simple and low-cost method to detect TEL in mice drinking water.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Khan A, Khan BT, Qayyum A. Comparative study of telmisartan with pioglitazone on insuline resistance in type 2 diabetic mice. *Pak Armed Forces Med J*. 2017;67(1):31-36.
2. Ikejima H, Imanishi T, Tsujioka H, et al. Effects of telmisartan, a unique angiotensin receptor blocker with selective peroxisome proliferator-activated receptor-gamma-modulating activity, on nitric oxide bioavailability and atherosclerotic change. *J Hypertens*. 2008;26(5):964-972.
3. Benson SC, Pershadsingh HA, Ho CI, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with

selective PPARgamma-modulating activity. *Hypertension*. 2004;43(5):993-1002.
4. Aubert G, Burnier M, Dulloo A, et al. Neuroendocrine characterization and anorexigenic effects of telmisartan in diet- and glitazone-induced weight gain. *Metabolism*. 2010;59(1):25-32.
5. Fujisaka S, Usui I, Kanatani Y, et al. Telmisartan improves insulin resistance and modulates adipose tissue macrophage polarization in high-fat-fed mice. *Endocrinology*. 2011;152(5):1789-1799.
6. Unger T, Schupp M. Telmisartan: from lowering blood pressure to end-organ protection. *Future Cardiol*. 2005;1(1):7-15.
7. Wienen W, Entzeroth M, van Meel JCA, et al. A review on telmisartan: a novel, long-acting angiotensin II-receptor antagonist. *Cardiovas Drug Rev*. 2000;18(2):127-154.
8. Destro M, Cagnoni F, Dognini GP, et al. Telmisartan: just an antihypertensive agent? A literature review. *Expert Opin Pharmacother*. 2011;12(17):2719-2735.
9. Galzerano D, Capogrosso C, Di Michele S, et al. New standards in hypertension and cardiovascular risk management: focus on telmisartan. *Vasc Health Risk Manag*. 2010;6:113-133.
10. Müller-Fielitz H, Hübel N, Mildner M, Vogt FM, Barkhausen J, Raasch W. Chronic blockade of angiotensin AT1 receptors improves cardinal symptoms of metabolic syndrome in diet-induced obesity in rats. *Br J Pharmacol*. 2014;171(3):746-760.
11. Shang F, Zhang J, Li Z, et al. Cardiovascular protective effect of metformin and telmisartan: reduction of PARP1 activity via the AMPK-PARP1 cascade. *PLoS One*. 2016;11(3):e0151845.
12. Sato-Horiguchi C, Ogawa D, Wada J, et al. Telmisartan attenuates diabetic nephropathy by suppressing oxidative stress in db/db mice. *Nephron Exp Nephrol*. 2012;121(3-4):e97-e108.
13. Ushijima K, Takuma M, Ando H, et al. Effects of telmisartan and valsartan on insulin sensitivity in obese diabetic mice. *Eur J Pharmacol*. 2013;698(1-3):505-510.
14. Krueger F, Kappert K, Foryst-Ludwig A, et al. AT1-receptor blockade attenuates outward aortic remodeling associated with diet-induced obesity in mice. *Clin Sci (Lond)*. 2017;131(15):1989-2005.
15. Graus-Nunes F, Marinho TS, Barbosa-da-Silva S, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Differential effects of angiotensin receptor blockers on pancreatic islet remodelling and glucose homeostasis in diet-induced obese mice. *Mol Cell Endocrinol*. 2017;439:54-64.
16. Qin Z, Niu W, Tan R. Spectrophotometric method for the determination of telmisartan with congo red. *J Anal Chem*. 2008;64:449-454.
17. Park J, Cho W, Cha KH, Ahn J, Han K, Hwang SJ. Solubilization of the poorly water soluble drug, telmisartan, using supercritical anti-solvent (SAS) process. *Int J Pharm*. 2013;441(1-2):50-55.
18. Yang L, Shao Y, Han HK. Improved pH-dependent drug release and oral exposure of telmisartan, a poorly soluble drug through the formation of drug-aminoclay complex. *Int J Pharm*. 2014;471(1-2):258-263.
19. Patel K, Dhudasia K, Patel A, Dave J, Patel C. Stress degradation studies on telmisartan and development of a validated method by UV spectrophotometry in bulk and pharmaceutical dosage forms. *Pharm Methods*. 2011;2(4):253-259.
20. Chavhan V, Lawande R, Salunke J, Ghante M, Jagtap S. UV spectrophotometric method development and validation for telmisartan in bulk and tablet dosage form. *Asian J Pharm Clin Res*. 2013;6(4):19-21.
21. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *J Pharmacol Pharmacother*. 2010;1(2):94-99.
22. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*. 1993;123(11):1939-1951.
23. Araki K, Masaki T, Katsuragi I, Tanaka K, Kakuma T, Yoshimatsu H. Telmisartan prevents obesity and increases the expression of uncoupling protein 1 in diet-induced obese mice. *Hypertension*. 2006;48(1):51-57.
24. Patel N, Patel JK. Analytical methodologies for determination of telmisartan: an overview. *Int J Pharm Pharmaceut Sci*. 2013;5(1):17-22. https://www.semanticscholar.org/paper/ANALYTICAL-METHODOLOGIES-FOR-DETERMINATION-OF-AN-Patel-Patel/4cb13653adff3ef638424dcbdadf5e93851b06cd. Accessed September 8, 2020.
25. Park C, Meghani NM, Shin Y, et al. Investigation of crystallization and salt formation of poorly water-soluble telmisartan for enhanced solubility. *Pharmaceutics*. 2019;11(3):102.
26. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J*. 2008;22(3):659-661.
27. Verdecchia P, Angeli F, Gentile G, Mazzotta G, Reboldi G. Telmisartan for the reduction of cardiovascular morbidity and mortality. *Expert Rev Clin Pharmacol*. 2011;4(2):151-161.
28. Kraly FS. Physiology of drinking elicited by eating. *Psychol Rev*. 1984;91(4):478-490.
29. Ellacott KL, Morton GJ, Woods SC, Tso P, Schwartz MW. Assessment of feeding behavior in laboratory mice. *Cell Metab*. 2010;12(1):10-17.
30. Zhang L. Voluntary oral administration of drugs in mice. *Protocol Exchange*. https://doi.org/10.1038/protex.2011.236.
31. Additives to the drinking water for rats and mice. Institutional Animal Care and Use Committee, 2014. Available from https://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/additives-to-the-drinking-water-for-rats-and-mice-iacuc/. Accessed September 8, 2020.
32. Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG. Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behav Genet*. 2002;32(6):435-443.
33. Pereira-Silva DC, Machado-Silva RP, Castro-Pinheiro C, Fernandes-Santos C. Does gender influence cardiovascular remodeling in C57BL/6J mice fed a high-fat, high-sucrose and high-salt diet?. *Int J Exp Pathol*. 2019;100(3):153-160.
34. Viera MC, Fernandes-Santos C, TdS Faria, Aguila MB, Mandarim-de-Lacerda CA. Diets rich in saturated fat and/or salt differentially modulate atrial natriuretic peptide and renin expression in C57BL/6 mice. *Eur J Nutr*. 2012;51(1):89-96.
35. Abdulla MH, Sattar MA, Johns EJ. The Relation between fructose-induced metabolic syndrome and altered renal haemodynamic and excretory function in the rat. *Int J Nephrol*. 2011;2011:934659.
36. Salama I. Simultaneous HPLC-UV analysis of telmisartan and hydrochlorothiazide in human plasma. *Bull Facul Pharm, Cairo Univ*. 2011;49(1):19-24.
37. Chen B-M, Liang Y-Z, Wang Y-L, et al. Development and validation of liquid chromatography-mass spectrometry method for the determination of telmisartan in human plasma. *Anal Chim Acta*. 2005;540(2):367-373.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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