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Validation of a rat behavioral avoidance model from a drug delivery perspective

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Abstract

Conventional taste-masking strategies are used to overcome the bitter taste perception of pharmaceuticals by coating the drug particles and/or adding flavoring agents. However, for certain product categories such as rapid dissolve sublingual tablets, taste-masking is challenging. Programs exploring such formulation strategies in the LO–CS phase or post CS phase possess very little toxicological information available in order to conduct human taste panel studies. The potential of a bitter taste perception can present a significant business risk. The objective of the study was to validate a rat behavioral avoidance model that identifies bitter-tasting compounds. Most classic bitter substances elicit a response in the micromolar concentration range while most drugs elicit a response in the millimolar range, hence a validation exercise was conducted to examine if the existing biological model was sensitive enough to identify known bitter tasting drugs as such.

Five compounds: ergotamine tartrate, fluoxetine, sucrose, sumatriptan and povidone were chosen to represent a spectrum of compounds. The bitter tasting compounds were identified as such in the model. Based on these results, the assay may serve as a useful surrogate test to identify compounds that may have bitter taste issues.

Keywords: Sumatriptan; Fluoxetine; Ergotamine; Bitter taste; Rat model; Migraine therapy

1. Introduction

The acceptance of some pharmaceutical products has been adversely affected by the presence of a bitter taste. Oral pharmaceutical dosage forms perceived to have bitter taste might include chewable and non-chewable tablets, capsules, syrups, suspensions, concentrates, lozenges, dentrifices, mouthwashes,

Abbreviations: CS, candidate selection; IC_{50}, concentration causing a 50% decrease in licking frequency; LO, lead optimization; SOA, sucrose octaacetate

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atomized solid or liquid inhalants, and ingestible ointments (Roy, 1994). Poor compliance and poor patient acceptance is especially an issue with pediatric and veterinary drugs and formulations (Thombre, 2004). Conventional taste-masking strategies include addition of flavor, coatings, use of lipophilic or hydrophilic vehicles, and preparation of sparingly soluble salts (Gregory et al., 1990). Rapid dissolve sublingual tablet formulations are gaining popularity as they present an advantage of offering a rapid absorption and may be suitable for pharmacological indications where a quick relief is needed or when the drug is subjected to extensive first pass effect. These are suitable for ambulatory use as they need not be ingested with a glass of water as with other conventional oral dosage formulations. However, formulations such as rapid dissolve sublingual tablets present a high risk factor for bitter tasting drugs as it not feasible to employ conventional taste-masking strategies. Additionally, the formulation lies in close proximity to the tastebuds. Conventional coating techniques cannot be employed, as these will decrease the rate of dissolution of the drug in the oral cavity.

Candidate molecules in the LO phase being expressly pursued for development as rapid dissolve sublingual products present challenges in terms of the conduction of a human taste panel study due to lack of sufficient toxicological information to make a study feasible in a timely manner. The rat behavioral avoidance taste model is based on the principle that presentation of a bitter solution to water-deprived rats reduces the drinking frequency (Boughter et al., 2002). Most bitter substances lead to a well-preserved avoidance mechanism in most species as the avoidance mechanism has evolved to help differentiate between nutrients and noxious substances (Herness and Gilbertson, 1999). In the model, rats are trained to drink water at a certain frequency and the concentration of drug causing a 50% drop in licking frequency compared to that of water is calculated.

For the model validation, certain marketed compounds were chosen that were known to present bitter taste issues. In a clinical trial with sumatriptan nasal spray, 24.5% patients complained of a bad or unusual taste when employing the 20 mg nasal spray formulation (product information on company website). Ergotamine tartrate and fluoxetine were chosen similarly based on product histories of the marketed compounds (product information on company website). Additionally, povidone and sucrose were chosen to represent neutral and good-tasting compounds, respectively. The five compounds were supplied in a blinded fashion to the investigator and the study conducted based on a 5-mg dose with an end concentration of 1 mg/mL in the oral cavity (Hansen et al., 1992). Cycloheximide, a reference bitter substance was employed for comparison in the study.

The study demonstrated that ergotamine, fluoxetine and sumatriptan caused a dose-dependent decrease in licking frequency that was accompanied with other avoidance behaviors such as grooming, retreating movements and jaw-smacking. The IC_{50} fell in the millimolar to sub-millimolar range. Though the IC_{50} for the bitter drugs were found to be two to three orders of magnitude below that of classic reference bitter substances such as cycloheximide, the model was able to predict and identify bitter compounds as such. Thus, the rat behavioral avoidance model may be employed as a surrogate tool to identify potential bitter-tasting compounds. Such an assay will be especially valuable for assessing the risk associated with bitter taste issues. The risk can be presumed to be high particularly when the IC_{50} falls in the range of the anticipated salivary concentration based on anticipated dose.

2. Materials and methods

2.1. Materials

All compounds were obtained in-house at Eli Lilly and Company with the exception of ergotamine tartrate (Fluka, St. Louis, MO).

2.2. Animals

Adult male Sprague–Dawley rats (250–300 g) were employed for the study.

2.3. Methods

Rats were water deprived and trained to drink in a specialized testing chamber (“the Davis rig”; MS-80; DiLog Instruments, Tallahassee, FL) that permitted brief access to water and test solutions and automatic collection of lick activity data.

2.3.1. Training

Rats were water deprived for ∼22 h and on the first day, after which they were placed in the Davis rig and given access to water presented in front of a continuously open shutter for 30 min. Rats were returned to their cages and given access to water for an additional 15 min to allow them to rehydrate before water was removed again. On the second day, rats were returned to the Davis rig for additional training that consisted of opening the shutter for 5 min followed by closed shutter periods for 1 min. The 5 min/1 min cycles were continued for a total of 30 min of shutter open time. The rats were then allowed to rehydrate before water was removed overnight. On the third and final training day, rats were tested using a sequence of water presentations that mimicked the subsequent testing days. Up to 70 test cycles were presented each lasting 8 s (shutter open). These test periods were alternated with 2-s rinse periods (to mimic water rinse periods), before moving to the next test solution. Rats were considered successfully trained if they licked successfully for a minimum of 40 consecutive test periods. Typically, eight rats entered training and the best-trained six were used for subsequent testing. Rats were always allowed to rehydrate after testing and never fell below 80% of their pre-deprivation weights.

2.3.2. Testing

To test for the behavioral responses to the unknown compounds, the following concentrations of two unknown compounds were made for each experiment daily: 0.005, 0.01, 0.05, 0.1, 0.5 and 1 g/L. The pH of the solutions was measured using a pH meter (Accumet model 25; Fisher Scientific) and osmolarity of solutions was verified with a vapor pressure osmometer (Vapro model 5520; Wescor, Logan, UT). In addition, two moderate concentrations of known bitter compounds (cycloheximide, denatonium) were prepared and included in the panel of test stimuli. The final two test chambers contained water, which served as the baseline for analysis of the other test compounds and served as the rinse between test stimuli.

On the fourth day, each rat was placed in the Davis rig and run through a sequence of test solutions presented randomly. Each test solution was presented for 8 s twice during the experiment. All test trials were interspersed with 2-s water rinse trials. The Davis rig was interfaced to a computer to record the number of licks during each stimulus presentation. In addition, the animals were videotaped to document other behaviors associated with bitter tastants (jaw smacking, oral grooming, withdrawal). These behaviors were not analyzed in the present study.

2.3.3. Data collection

Data was obtained from a group of six rats per group. Data were averaged within each rat for the presentation of each stimulus and each concentration. The average number of licks was then divided by the average number of licks during the water presentation to generate the % inhibition of licking as follows:

$$\%\ \text{inhibition of licking} = \frac{\text{mean number of licks to stimulus}}{\text{mean number of licks to water}} \times 100$$

The % inhibition of licking generated for each animal and each concentration was then averaged across animals to generate the concentration–response functions shown in the data for each of the five unknown compounds and one known bitter (aversive) compound, cycloheximide. Data were fit with a logistic function (Origin v. 6.1) to determine the IC_{50}, or the concentration producing a half-maximal inhibition of licking activity (relative to water).

3. Results

Compounds 1, 2 and 5 supplied blinded (ergotamine tartrate, fluoxetine and sumatriptan, respectively) showed a concentration-dependent decrease in licking behavior (Figs. 1, 2 and 5), while compounds 4 and 6 (sucrose and povidone) were not seen to elicit any inhibition of licking activity relative to water (Figs. 4 and 5). The IC_{50}s were two to three orders of magnitude below response to cycloheximide (stippled bar in Figs. 1–5 for comparison). Cycloheximide, a known intensely bitter stimulus to rats, elicited a similar concentration-dependent inhibition of licking activity (see Fig. 6). Consistent with the concentration-dependent decrease in licking, the behavioral responses to ergotamine, fluoxetine and sumatriptan included jaw-smacking, grooming and retreating movements. The behavior was more pronounced to higher concentrations of above test substances (personal

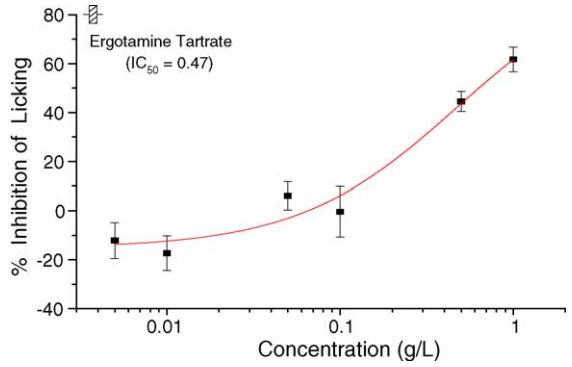

Fig. 1. Response to ergotamine tartrate. Stippled bar denotes response to cycloheximide. Bars denote S.D., $n = 6$.

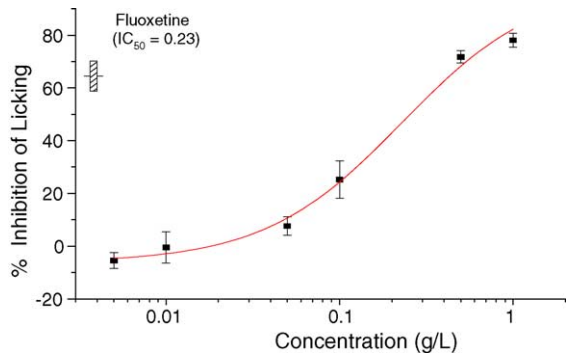

Fig. 2. Response to fluoxetine. Stippled bar denotes response to cycloheximide. Bars denote S.D., $n = 6$.

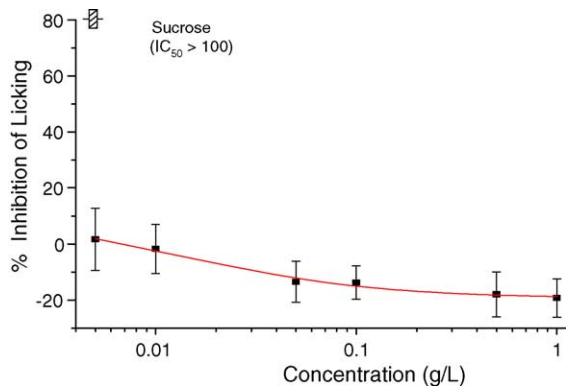

Fig. 3. Response to sucrose. Stippled bar denotes response to cycloheximide. Bars denote S.D., $n = 6$.

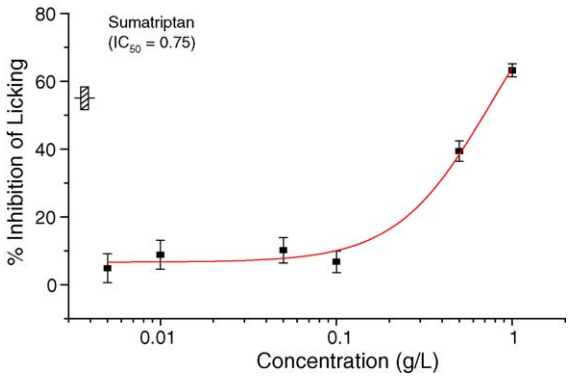

Fig. 4. Response to sumatriptan. Stippled bar denotes response to cycloheximide. Bars denote S.D., $n = 6$.

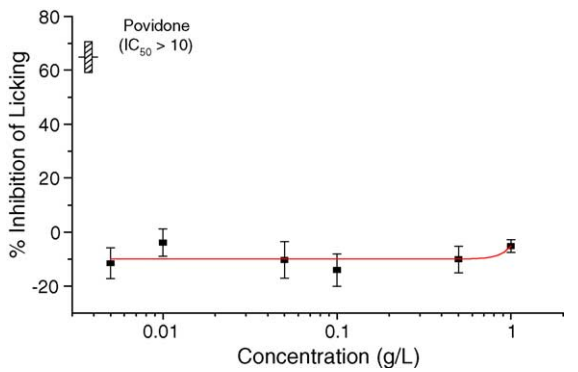

Fig. 5. Response to povidone. Stippled bar denotes response to cycloheximide. Bars denote S.D., $n = 6$.

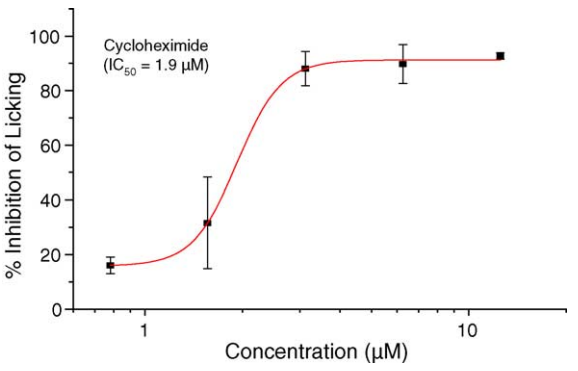

Fig. 6. Response to cycloheximide. Bars denote S.D., $n = 6$.

observation). The range of concentrations tested for the bitter tasting drugs elicited a partial sigmoidal response in comparison with the curve for cycloheximide as a broader range of concentrations was not tested. The rank ordering of bitterness was found to be ergotamine tartrate > fluoxetine > sumatriptan based on molar concentrations.

4. Discussion

The brief-access taste test as conducted above has been found to be more accurately reflective of gustatory processing (St. John et al., 1994) than other models such as the solution preference or avoidance test, which is measured by fluid intake involving tests that last 24–48 h (Nejad, 1986; Smith, 1988). Brief-access taste tests involve the presentation of a taste stimulus for durations between 5 and 30 s and the dependent measure is the number of licks an animal makes in trial (Grill et al., 1987). In brief-access tests with aversive stimuli, water deprivation is commonly used to motivate licking behavior, and to provide a baseline of licking from which a concentration-dependent decrease can be measured. Depending on the testing apparatus, multiple concentrations of a taste stimulus may be presented, and a concentration–response function for an individual animal may be obtained. The brief-access test can be used to obtain data in a timely manner and will lessen the influence of post-ingestive behaviors. Strains of mice were found to avoid phenylthiocarbamide in two-bottle intake tests after a few days of consumption (Whitney and Harder, 1986). This was attributed to taste conditioning in response to mild toxic effects. In a study, the licking behavior to sucrose octaacetate (SOA) was found to be concentration dependent for SW and T mice strains while C3 and D mice were indifferent. This finding was consistent with the genetic makeup of SW and T mice as the sensitivity to SOA is determined by allelic variation at a single genetic locus on mouse chromosome 6 (Warren and Lewis, 1970; Lush, 1981). During preference testing, SW mice consumed more total fluid than other three strains during a 48 h test (Boughter et al., 2002). The ratio method to determine the % inhibition additionally corrects for differences in an animal's ability to make a certain number of licks in a given trial. The method also offers microstructural details of licking patterns in the training period, the immediate behavioral response, the latency to first lick and time between licks. The water rinse period of 2-s was found to be adequate to minimize carry over effects from the previous taste stimulus presented. The pH of the measured solutions was above pH 4.5, which is the threshold for perception of a sour taste, which can also induce an aversive response (Gilbertson and Gilbertson, 1994).

Ergotamine and sumatriptan are antimigraine compounds that are well known to have taste issues associated with them. Ergotamine belongs to the family of Ergot alkaloids and presents the classic bitter taste most plant alkaloids present. Ergotamine and sumatriptan are marketed as fast-dissolve tablets and nasal sprays (Migranal® and Imitrex® brand, respectively). In a clinical study for Imitrex® nasal spray, 13–19–24% patients treated with the 5, 10 or 20 mg dose, respectively, complained of a bad taste perception (data on product information website). Migraine patients reported that the most desirable attribute in a product is quick relief and hence nasal sprays and fast-dissolve tablets offer an attractive alternative. Migraineurs additionally present gastric stasis leading to erratic and protracted absorption profiles via the oral route (Cipolla et al., 2001). The IC_{50} values for fluoxetine, ergotamine tartrate and sumatriptan were found to be less than 1 g/L, which was the anticipated salivary concentration, based on a 5 mg dose. Hence, in retrospect the rat behavioral assay could have potentially predicted the taste perception issues reported by patients in the clinic. Cycloheximide, the reference bitter substance presents an IC_{50} in the micromolar range while the bitter drugs studied presented millimolar to sub-millimolar IC_{50} concentrations. Though the IC_{50}s were two to three orders of magnitude higher than for reference bitter substances usually employed, the assay was able to register the decrease in licking frequency. The videotaped behavior also displayed other hallmarks of aversive response such as jaw-smacking and grooming.

The usefulness of such a prediction for molecules in LO phase will help to calculate risk associated with taste perception and also potentially allow for the consideration of the increased cost of the use of taste-masking strategies, where feasible. Povidone is polyvinyl pyrrolidone, an inert, neutral tasting excipient employed as a film former and plasticizer. The lack of a response to povidone at low concentrations demon-

Table 1
Calculated IC_{50} values for compounds in comparison to cycloheximide

Test compound	Calculated IC_{50} (g/L or molar concentration)
#1 (Ergotamine tartrate)	0.47 g/L (0.36 mM)
#2 (Fluoxetine)	0.23 g/L (0.66 mM)
#4 (Sucrose)	>100 g/L
#5 (Sumatriptan)	0.75 g/L (2.54 mM)
#6 (Povidone)	>10 g/L
Cycloheximide	0.534 mg/L (1.9 μM)

strates the ability of the test to distinguish between pharmaceutical drugs and excipients. Sucrose is a nutritive and most rats did not show a decrease in licking frequency up to the highest concentration employed in this test.

5. Conclusions

Ergotamine tartrate, fluoxetine and sumatriptan were found to elicit aversive responses in water-deprived rats; consistent with those types of responses generated by known bitter stimuli such as cycloheximide. These responses were scored in the anticipated exposure concentration based on the dose. These compounds exhibited aversive behaviors such as jaw-smacking, and retreating movements, similar in response to cycloheximide. The model classified these compounds as bitter though the IC_{50} of the drugs was two to three orders of magnitude higher than the classic bitter reference, cycloheximide (Table 1). The identification of drugs known to have bitter taste issues by the model validates it as a useful model to predict bitter taste issues. The bitter taste may be an issue if the IC_{50} lies in the range of the anticipated exposure concentration. The model may be additionally employed to rank order compounds. The model may be employed as an early surrogate test to anticipate bitter taste issues and may offer some insight on the potential risk involved based on the anticipated salivary concentration and the IC_{50} values obtained.

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