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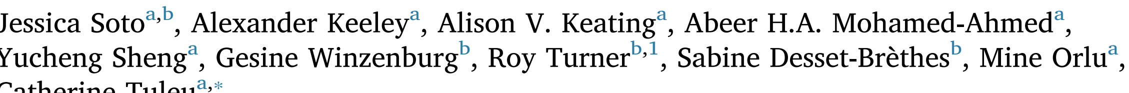
Research paper

Rats can predict aversiveness of Active Pharmaceutical Ingredients



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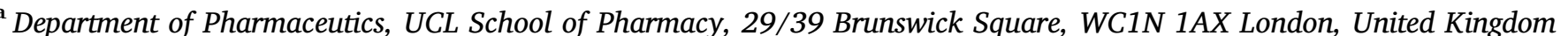

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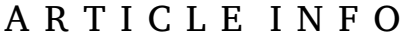

A R T I C L E I N F O

Keywords:
Taste assessment
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A B S T R A C T

Taste is crucial for patient acceptability and compliance with prescribed medicines, in particular with pediatric patients. Evaluating the taste of new active pharmaceutical ingredients (APIs) is therefore essential to put in place adequate taste-masking techniques, if needed, which will lead to acceptable palatable formulations. Thus, there is an urgent need to develop and optimize taste assessment methods that could be used at different stages of the drug development process. The aim of this study was to investigate the suitability of the rat brief-access taste aversion (BATA) model as a screening tool for assessment of APIs aversiveness that could predict human taste responses. Presently, the taste intensity of nine marketed APIs known to have different levels of bitter intensity (quinine hydrochloride dihydrate, 6-n-propylthiouracil, sildenafil citrate, diclofenac sodium, ranitidine hydrochloride, caffeine citrate, isoniazid, telbivudine and paracetamol) was investigated at different overlapping concentrations with two *in vivo* taste assessment methods: the rat BATA model and human taste panels with the intention of determining the drugs' concentrations to produce half of the maximal rating. Overall there was a strong correlation ($R^2 = 0.896$) between rats IC_{50} and humans EC_{50} values. This correlation verifies the BATA model as a rapid and reliable tool for quantitative assessment of API aversiveness. A comparable ranking order was obtained mainly for high and medium aversive compounds, whereas it was less aligned for weakly aversive compounds. It was nonetheless possible to propose a classification of poor taste intensity determined in rats that would predict human taste tolerability.

1. Introduction

Taste assessment studies have indirectly become essential during pharmaceutical development of pediatric medicines due to pediatric regulations in the United States and European Union [1].

Evaluating the taste of different derivatives of new chemical entities (NCE) such as salts and isomers during the early stages of the drug development process is therefore of utmost importance to identify taste aversive compounds at screening stages of pharmaceutical development and optimize taste-masking strategies to improve patient adherence and acceptance. Thus, there is a great interest to develop and verify a robust method to assess the taste of pharmaceutical compounds at early stages of drug development.

Taste can be assessed with both *in vivo* and *in vitro* techniques. The most widely used and gold standard method to evaluate the taste is by a human taste panel. However, this method presents many challenges since the taste assessment of NCEs can only be performed if sufficient toxicological data are available in humans. Unfortunately at the early stages of the drug development process, toxicological data in humans are extremely limited or nonexistent. Therefore, human taste data would not usually be available at the time of writing the Pediatric Investigation Plan (PIP) and the final pediatric dose(s) may not be defined until later in the development program [2]. This will result in very limited information about pediatric dosage forms in the PIP whose selection is often influenced by taste-masking opportunities and palatability. Moreover, ethical and/or safety approval can limit human taste

Abbreviations: ADI, Acceptable Daily Intake; API, Active Pharmaceutical Ingredient; BATA, Brief-Access Taste Aversion; CI, Confidence Interval; EC_{50}, Concentration of the drug that produces half of the maximal rating (1 0 0) in the human taste panels; GMP, Good Manufacturing Practice; IC_{50}, Concentration which suppresses 50% of the licks compared to the reference; NCE, New Chemical Entity; PIP, Pediatric Investigation Plan; REC, Research Ethic Committee; SOP, Standard Operating Procedure; T2R, type 2 taste receptor; VAS, Visual Analogue Scale

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panels. For example, for several drugs, e.g. cytotoxics, it would be considered unethical to enroll healthy volunteers, even in swirl and spit tests. In addition, taste panels are very costly; the training of adult volunteers and the overall cost for running and setting up a human taste panel is expensive. Taste trials are also time-consuming and very laborious, which can result in taste fatigue of the volunteers and inaccuracy of the results. The large taste perception variability between individuals is also another drawback of human taste panels.

Other approaches such as animal models and taste sensors (e-tongues) have shown early promises and optimization of these techniques was encouraged [3] including industrial application. Among these approaches, the rodent BATA model has great potential and has already shown promising results [4–8]. The rodent BATA model is a relatively simple and fast method to detect successfully in an objective and quantitative manner the aversive taste of structurally different APIs. In this animal model, mice or rats, are mildly water-deprived and then put into a special experimental cage. The cage records the number of licks (lickometer) that the rodents make if the API is presented in different concentrations in several sipper tubes. Animals only have a very short period of time (between 5 and 10 s) to test each solution. Typically, a high number of licks indicate a pleasant taste whereas a low number of licks indicate an aversive taste. With this procedure, a full dose–response (concentration–aversion) curve of lick rate can be obtained over a short period of time with very few animals.

Devantier et al. [4] showed that the relative potencies of four drugs (quinine, ciprofloxacin, clarithromycin, and nystatin) assessed with the mouse BATA assay (n = 16–18) correlated well with the taste intensities evaluated by a trained human taste panel (n = 10). Rudnitskaya et al. [7] found exactly the same rank order of bitterness prediction for eight drugs (azelastine hydrochloride, caffeine, chlorhexidine digluconate, potassium nitrate, naratriptan hydrochloride, paracetamol, quinine hydrochloride and sumatriptan succinate) assessed with the rat BATA model (n not specified) and a trained human panel (n = 15). The rat data showed exactly the same rank order of bitterness prediction as the human panel with a consistent offset of approximately half-log unit of molar concentration, with rats always rating the bitterness lower than humans. They explained this offset by the fact that rats were encouraged to drink whilst the human panel was not. Noorjahan et al. [9] assessed the taste of an iron EDTA complex dissolved in water as well as formulated in chewable and orodispersible tablets with a human taste panel (n = 6) and a rat BATA model (n = 6). They found that the correlation coefficient between mean responses of rats and humans was above 0.5 and concluded a good correlation. However, the methodology Noorjahan et al. (2014) used for the taste assessment with rats was different from the BATA procedure usually described in previous published studies [9]. Rats were water-deprived for 24 h and were then presented to a bottle containing water for 5 min; the licking activity was taken as standard. After similar water-deprivation duration, rats were randomly presented to three different concentrations of the drug under assessment. The number of licks taken in five minutes was counted and a percentage of licking frequency compared to water was calculated. This made any cross comparison between studies difficult.

Moreover, additional testing in humans, using other compounds was encouraged by Devantier et al. [4] to ascertain and complete the data available in the literature showing that the rodent BATA model is predictive of human taste data.

Therefore the aim of this study was to conduct a well-designed prospective rat BATA experiments and human taste panels with approved compounds. Nine APIs of various levels of aversiveness (i.e. known for their high to weaker bitterness intensity in human) have been selected in order to investigate systematically if strong correlation between both *in vivo* taste assessment techniques could be established. This would position the rat BATA model as a taste assessment tool that could be used during drug development even at an early stage.

2. Material and methods

2.1. Taste solutions

For the rat BATA experiments, quinine hydrochloride dihydrate, ranitidine hydrochloride, isoniazid, paracetamol and diclofenac sodium were purchased from Sigma Aldrich (Sigma Aldrich, Dorset, UK). Caffeine citrate and 6-n-propylthiouracil were bought from Fagron (Fagron, Newcastle upon Tyne, UK) and sildenafil citrate was obtained from PLIVA d.o.o. (Zagreb, Croatia). For the human taste panels, quinine hydrochloride dihydrate, paracetamol, diclofenac sodium, 6-n-propylthiouracil, ranitidine hydrochloride were purchased from Fagron (Fagron, Newcastle upon Tyne, UK). Caffeine citrate and sildenafil citrate were purchased from Guinama (Guinama, Valencia, Spain). Isoniazid was obtained from Macleods Pharmaceuticals (Mumbai, India). Telbivudine was produced and given by Novartis for both experiments. For the human studies all the drugs were of pharmaceutical grade Good Manufacturing Practice (GMP) compliant.

For each drug, series of up to six concentrations were tested with the rat BATA experiments. Up to four concentrations of these were selected for the human taste panels in order to have concentrations that overlapped on the two *in vivo* panels. The concentration levels for the two types of taste assessment are listed in Table 1. The different solutions for each drug were prepared by dilutions from the stock solution prior to each taste assessment session in Buxton® natural mineral water (Buxton, UK) for the human taste panels and in deionized water for the rat BATA experiments. The stock solution for each drug was prepared by dissolving the amount of the corresponding API in a fixed volume of water under magnetic stirring. Sonication at room temperature was applied to facilitate dissolution determined by visual inspection of APIs when needed. All the solutions were tested at room temperature. For the human taste panels, all extemporaneous preparations were carried out under the supervision of a registered UK pharmacist following Standard Operating Procedures (SOPs).

2.2. Rat BATA model

2.2.1. Animals

A total of 20 naive adult male Sprague–Dawley rats (Charles-River, Kent, UK) were used for these experiments. A cohort of n = 10 rats was used for each BATA experiment. Upon arrival rats received a minimum of 7 days of acclimatization to the new environment. They were housed in pairs in standard cages in a room that was maintained at 21 ± 2 °C with 55 ± 10% humidity and with a 12:12 h light/dark cycle. All training and testing occurred during the light phase of the cycle. Animals had free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a water-restriction schedule occurred. Throughout the experiments, daily food and water consumption were monitored. As a safety and welfare measure it was checked that their weight did not drop below 85% of their free-feeding weight. A washout-period of one week was respected between the different BATA experiments as the rats become naïve again after this time period and can be used again. All the procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project License PPL 70/7668).

2.2.2. BATA procedure

The commercially available lickometer “Davis MS-160” from DiLog Instruments (Tallahassee, Florida, USA) was used for these experiments. Each rat was water-deprived for 22 h before each session (training and testing) and was then placed in the lickometer for a maximum session-length of 40 min. After each session, the rodents received tap water for rehydration for 1 h after experimental day. The first days of the protocol were dedicated to training: on the first training day the shutter was continually open, presenting a single tube containing deionized water; on the second training session the sixteen tubes contained deionized

Table 1
APIs selected and their respective therapeutic use and concentrations (mM) in the rat BATA experiments and human taste panels.

API	BATA experiments concentrations (mM) and human taste panels concentrations (in BOLD)	Solubility in water (mM)
Quinine hydrochloride dihydrate	0.01 **0.03 0.1 0.3 1** 3	157.5
6-n-propylthiouracil	**0.03 0.3 2.9**	6.5
Sildenafil citrate	0.1 **0.2 0.4 1.3 2.5** 5	6.1
Diclofenac sodium	0.03 **0.1 0.3 1 3** 10	157.2
Ranitidine hydrochloride	0.2 0.4 **0.8 1.6 3.2 4.8**	5.1
Caffeine citrate	**0.3 1 3 10** 30 100	245.9
Isoniazid	9.1 **18.2 36.5 72.9 145.8 291.7**	911.5
Telbivudine	**4.1** 10.3 **20.6 41.3** 61.9 **82.6**	> 82.6
Paracetamol	3 **5 10 30 50** 90	92.6

water. The training was followed by two testing days during which each rat was presented with different sipper tubes containing either deionized water or one of the concentrations of the API under assessment. The trial began when the rat took its first lick from the sipper tube, and ended 8 s later when the shutter closed. A different sipper tube was positioned behind the shutter in preparation for the next trial during the inter-trial interval. Each trial was intercepted by a water rinse to minimize carry over effects from the previous solution tested. The order of presentation of the sipper tubes was randomized and each concentration was presented 4 times per session. This is an optimized procedure described in Soto et al. [10].

2.3. Human taste panel study

2.3.1. Participants

A total of twenty one healthy volunteers (10 females and 11 males, between the ages of 18 and 45 years; average age of the volunteers = 27 years) were recruited to conduct the taste assessment of

quinine hydrochloride dihydrate, sildenafil citrate, diclofenac sodium, caffeine citrate and paracetamol. Thirty one healthy volunteers (8 males and 23 females, between the ages of 18 and 38 years; average age of the volunteers = 23 years) were enrolled for the taste evaluation of 6-n-propylthiouracil, ranitidine hydrochloride and telbivudine. Twenty healthy adults (11 females and 9 males, in the age range 18–40 years; average age of the volunteers = 26 years) assessed the taste of isoniazid. All studies were randomized single blinded.

Healthy volunteers included in the studies were able to understand and speak English. If smokers, they had to forbear smoking at least one hour before and during all the sessions. Breakfast and neutral lunch (not spiced, lightly salted) were advised to be taken at least 30 min before the tests. Volunteers were excluded from the studies if they had deterioration of taste or smell, known drug allergies, underwent recent dental care or were taking a medical treatment (excluding contraceptives) up to 15 days before the tests.

The studies were conducted in accordance with the Declaration of Helsinki and its amendments, and the protocols were approved by the Research Ethics Committee at The School of Pharmacy, University College London.

2.3.2. Taste assessment

Single blind, cross over, single center studies were conducted to assess the taste of the nine APIs using the "swirl and spit" method. Each volunteer was given 10 mL sample solutions of different drug concentrations labelled with a randomized code. Subjects were instructed to swirl the content of each sample in their mouth for 5 s to cover all buccal surfaces and then spit it out into the plastic cup provided. Immediately after spitting, volunteers were asked to rate the taste intensity using a computerized questionnaire *Qualtrics* (Qualtrics, Provo, US) with 100 mm continuous Visual Analogue Scale (VAS) ranging from not aversive to extremely aversive. Before and after each sample, volunteers rinsed their mouth with Buxton® mineral water and could have low salt low fat crackers (Rakusen's Limited, Leeds, UK) to neutralize their palate. A minimum interval of 6 min (usually 10) was respected between samples until the previous sample could not be perceived anymore. Subjects were allowed to immediately re-taste each sample once, if needed.

The volunteers were asked to attend nine sessions (one session per drug), two hours in duration each. A minimum of 48-h (usually 1 week) washout-period was respected in between the sessions in order to reduce the burden on volunteers and minimize taste fatigue and contact with the test samples. One drug at up to four different concentrations was assessed per session. Each solution was given three times in a randomized order. In order to calibrate the panelists, a sample of bottled water (negative control) was given at the beginning of each session. In addition, quinine hydrochloride dihydrate at a concentration of 1 mM (positive control) was tested by the blinded volunteers during each session at a different time point within the randomization schedule. Each volunteer received the samples in the same randomized order. All volunteers tasted a total of 14 samples (4 concentrations of one drug, each tested 3 times and a positive and a negative control) per session. For each API, the taste intensity of each concentration was calculated for each volunteer by averaging the ratings obtained for the three replicates. The average ratings obtained from all the volunteers were then determined for each concentration.

2.4. Data treatment and analysis

The normality of the data was checked with the Shapiro-Wilk test for each API. As the data were not normally distributed, the Kruskal-Wallis test was performed to check if there were any differences in the number of licks among the different concentrations tested with deionized water. When significant, post-hoc analysis was carried out with Gao et al. non-parametric multiple test [11]. In order to rank the taste intensities of the different APIs an IC_{50} value which corresponds to the concentration of the drug that inhibits 50% of the maximum number of licks compared to the reference, deionized water, was calculated for each API with an E_{max} model [10,12].

The same statistical tests were performed for the human taste panels. Statistical analyses were also performed with the same tests described above to check if there were significant differences for the rating of the positive control between the different sessions and between volunteers. In order to compare the taste intensities obtained for the nine APIs, an EC_{50} value which corresponds to the concentration of the drug that produces half of the maximal rating (1 0 0) was calculated with the following E_{max} model:

$$E = \frac{E_{max} \times C^{Hill}}{EC_{50}{}^{Hill} + C^{Hill}} + \varepsilon$$

where E is the taste rating, E_{max} represents the maximum taste rating fixed to 100, C refers to the concentration of the drug, *Hill* is the slope factor affected by the gradient of the curve, EC_{50} represents the concentration which elicits a half-maximal taste rating and ε is the participant variability. EC_{50} values and 95% confidence interval (CI) were derived from this model for each API. Variance values for each of the parameters were also obtained as well as the error value, as a reflection of volunteer variability.

Correlation between rat taste data and human taste data was examined qualitatively by ranking the IC_{50} and EC_{50} values and quantitatively by plotting these values on a graph to determine a correlation factor.

All statistical analyses, boxplots generation and some graphs were done with R (version 3.0.1). Microsoft Office Excel 2010 was also used for the generation of graphs. IC_{50} and EC_{50} values were estimated by using the non-linear mixed effects modelling that was performed using the software NONMEM® (ICON, Ellicott City, Maryland, version 7.3) in conjunction with a gfortran (64-bit) compiler using Perl-Speaks NONMEM ® (PSN, version 4.2.0) as an interface to run NONMEM®.

3. Results

3.1. Rat BATA model

The number of licks was inhibited with increasing concentration for all nine APIs (Fig. 1). This demonstrated that the rats were able to detect the aversive taste of all the model drugs. Typically, a high number of licks indicate a pleasant taste whereas when licks are nearly completely suppressed it indicates an aversive taste (i.e. a noxious or punishing stimulus causing avoidance). However, an aversive response in the BATA model can be caused by an aberrant taste or flavor (e.g. bitter, but also sour, metallic, burning, spicy…) as well as other aversive physicochemical properties (e.g. pH, viscosity, grittiness). Purposefully only soluble compounds were used in this work for their known bitter taste attribute in human more or less intense.

The range of concentrations completely inhibiting the number of licks was covered for almost all the APIs tested. The concentrations chosen for quinine hydrochloride dihydrate, caffeine citrate and isoniazid enabled to produce a full concentration-response curve with a nearly complete inhibition of licks with the highest concentration (around 90% inhibition). 6-n-propylthiouracil, diclofenac sodium and sildenafil citrate inhibited 80% of the licks with the highest concentration. Ranitidine hydrochloride reduced the number of licks compared to water by nearly 60% at the highest concentration. Paracetamol only inhibited 38% of the licks compared to water at the highest concentration, 90 mM, which was the highest concentration that could be tested due to the solubility limit. Finally, telbivudine managed to decrease the number of licks compared to water by 35% only; a larger range of concentrations could have been explored for this API. The screening of the range of concentrations inhibiting the number of licks in rats was done first. This was done irrespectively of the

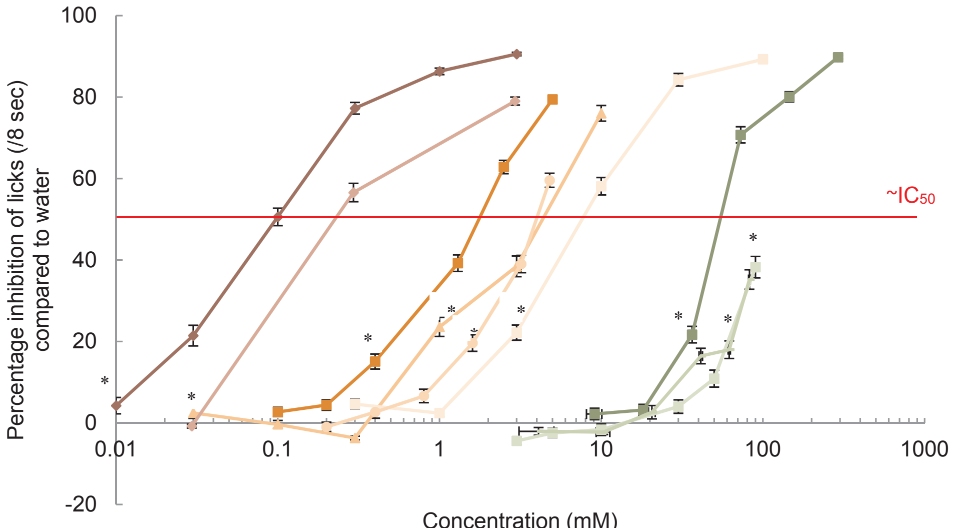

Fig. 1. Average percentage inhibition of licks (/8 s) (± SEM) compared to water for quinine hydrochloride dihydrate, 6-n-propylthiouracil, sildenafil citrate, diclofenac sodium, ranitidine hydrochloride, caffeine citrate, isoniazid, telbivudine, and paracetamol for n = 10 rats. *First concentration significantly different ($p < 0.05$) compared to the reference, deionized water. Significance based upon non-parametric Kruskal-Wallis test results using median.

therapeutic dose but starting close to maximum API solubility assuming that the maximum concentration that can be solubilized in water would be what the taste buds would be exposed to. For each API, four out of the six API concentrations tested in rats were also tested in the human taste panels to correlate the data head to head. For more accuracy API dissolved in saliva would be better however, saliva flow and composition (simulated vs un-simulated) varies therefore it is difficult to strictly mimic [13].

Quinine hydrochloride dihydrate and 6-n-propylthiouracil were the most aversive compounds (to the left of the graph).

A group of 4 compounds occupied the center of the graph (medium aversiveness).

Finally a group of 3 compounds sat on the right of the graph and were deemed the least aversive.

3.2. Human taste panel

For each drug, 63 data points were recorded per concentration averaging the data from all the volunteers (Fig. 2). As expected, the ratings increased with increasing concentrations for each drug. The volunteers were able to differentiate between the aversiveness of the nine drugs at different concentrations.

Briefly, for quinine hydrochloride dihydrate, 6-n-propylthiouracil, diclofenac sodium, ranitidine hydrochloride, isoniazid, telbivudine and paracetamol, all the concentrations were found to be significantly different from one another and the controls ($p < 0.05$) indicating the sensitivity of the panelists to the different concentrations of these APIs. Quinine hydrochloride dihydrate, 6-n-propylthiouracil and diclofenac sodium concentrations were separated by a three-fold increment, which seems to be an adequate interval for the separation of the concentrations that can be distinguished by humans. The concentrations chosen for paracetamol were not all following a three-fold increment. The two lowest concentrations of paracetamol were slightly different from each other with a p-value that was at the limit of the significance level ($p = 0.032$) and only seven volunteers were able to clearly differentiate 5 mM from 10 mM. This showed a reduced sensitivity of the panelists to small increments (two-fold increment) in concentrations that were low. Similarly, the two lowest concentrations of sildenafil citrate, 0.2 and 0.4 mM, were not significantly different from each other ($p = 0.168$). As for paracetamol, this could be due to the small increment between these two concentrations. It can be noticed from the graph that for all the volunteers, only the highest concentration was found to have an extremely aversive taste, similar to the quinine control with a p-value close to the level of significance ($p = 0.040$). All the concentrations of caffeine citrate were significantly different from one another ($p < 0.05$) and the highest concentration (10 mM) was rated as

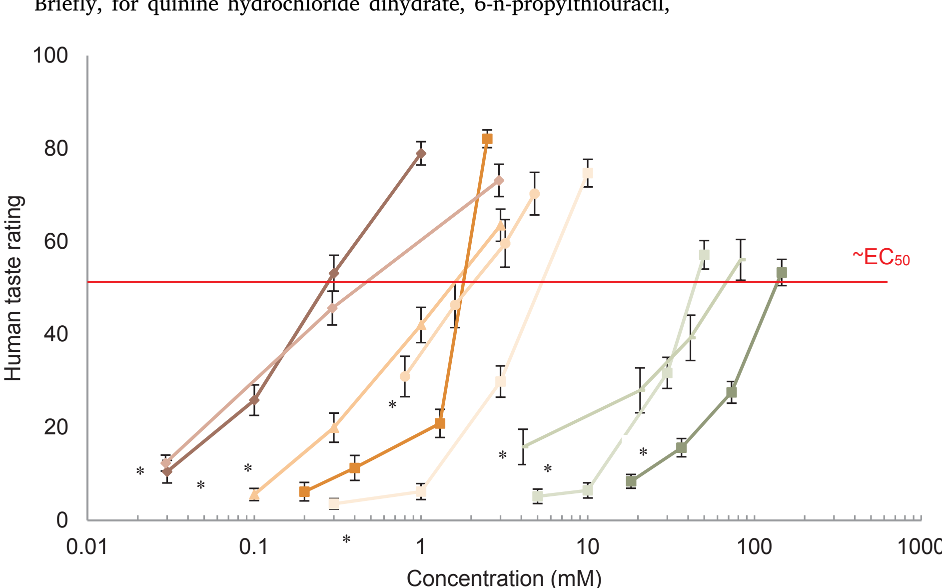

Fig. 2. Average taste ratings (± SEM) for quinine hydrochloride dihydrate, 6-n-propylthiouracil, sildenafil citrate, diclofenac sodium, ranitidine hydrochloride, caffeine citrate, isoniazid, telbivudine, and paracetamol for humans. *First concentration significantly different ($p < 0.05$) compared to the reference, mineral water. Significance based upon non-parametric Kruskal-Wallis test results using median.

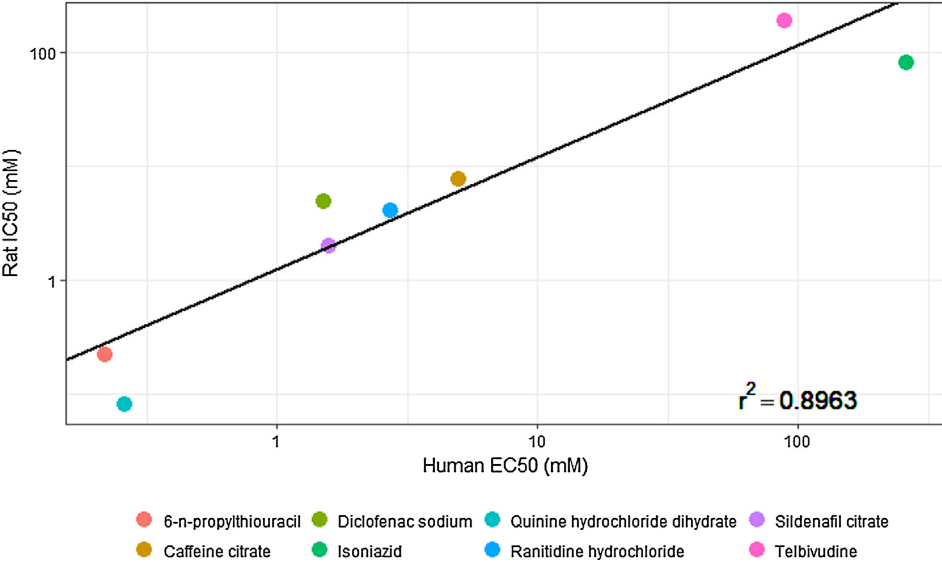

Fig. 3. *In vivo/in vivo* correlation plot between EC_{50} values (mM) for human panels and IC_{50} values (mM) for rat panels for 8 APIs.

aversive as the quinine control (1 mM) by the panel (p = 0.592).

3.3. Comparison of rat BATA experiments and human taste panels

Despite a slight shift between rat and human responses, a strong correlation ($R^2 = 0.8963$) was observed between the two panels (Fig. 3), the resulting equation being $IC_{50} = 1.0469(EC_{50})^{0.9101}$. The correlation plot of *in vivo/in vivo* taste intensities was done for eight APIs excluding paracetamol as no IC_{50} value could be obtained in rats for paracetamol. The IC_{50} and EC_{50} values were generated based on rat and human taste panels for the selected APIs, except for paracetamol and telbivudine where no IC_{50} value could be calculated in rats (Table 2). Rats assessed quinine hydrochloride dihydrate as the most aversive API followed by 6-n-propylthiouracil, sildenafil citrate, ranitidine hydrochloride, diclofenac sodium, caffeine citrate, isoniazid, telbivudine and paracetamol. This rank order is in agreement with the ranking predicted based on the nine concentration-response curves (Fig. 1). The human sensory data showed a similar rank order of aversive taste prediction as the rat BATA model for the high and medium aversive compounds (Fig. 3). However, for low aversive compounds, the IC_{50} ranking order between rats and humans slightly differs showing a lower correlation between the two taste assessment techniques (quinine hydrochloride dehydrate ~ 6-n-propylthiouracil > diclofenac sodium ~ sildenafil citrate > ranitidine hydrochloride > caffeine citrate, paracetamol > isoniazid > telbivudine in humans).

EC_{50} values obtained from the human taste studies were generally within less than one-half log unit of molar concentration of those derived from the rat BATA model (Table 2).

3.4. Proposed classification for prediction of human taste response based on rat BATA data

The proposed classification made to predict human taste response based on rat BATA data is shown on Table 3.

In order to verify the aforementioned, 4 compounds not included in the building proposed classification were used to calculate the EC_{50} from the IC_{50} (with equation $IC_{50} = 1.0469(EC_{50})^{0.9101}$). This calculated EC_{50} was then compared to the measured EC_{50}. The BATA and human taste panel methodologies used to generate the EC_{50} and IC_{50} were identical to the one described in this paper. Table 4 shows the outcome of this exercise.

For these 4 compounds the rat data were 100% predictive of the human level of aversiveness of the API. *Please note that despite quinine*

Table 2
Rat IC_{50} values and human EC_{50} values for quinine hydrochloride dihydrate, sildenafil citrate, diclofenac sodium, caffeine citrate and paracetamol in mM (n.d.: not determined; * this value was derived from a graph and is an approximation).

Drug	Rat IC_{50} and corresponding 95% CI (mM)	Human EC_{50} and corresponding 95% CI (mM)	Log IC_{50} – Log EC_{50}	Comparison with literature (IC_{50})	Comparison with literature (EC_{50})
Quinine hydrochloride dihydrate	0.08 (0.01–0.16)	0.26 (0.14–0.37)	−0.51	~0.1 (Rudnitskaya et al. [7])	~0.01 mM (calculated) for quinine hydrochloride; Rudnitskaya et al. [7] *0.658 mM for quinine sulfate; Devantier et al. [4]
6-n-propylthiouracil	0.22 (0.12–0.35)	0.22 (0.12–0.29)	0.00	/	/
Sildenafil citrate	2.00 (1.56–2.45)	1.58 (1–2)	0.10	/	/
Ranitidine hydrochloride	4.02 (2.82–5.22)	2.73 (1.96–4.40)	0.17	/	/
Diclofenac sodium	4.91 (1.17–10.99)	1.51 (1–2)	0.51	/	/
Caffeine citrate	7.76 (5.62–9.90)	5.01 (4–6)	0.19	~50 mM with caffeine base (Rudnitskaya et al. [7])*	~5 mM with caffeine base (Rudnitskaya et al. [7])*
Isoniazid	80.94 (54.93–106.95)	259 (80.05–437.95)	-0.51	/	/
Telbivudine	187.45 (74.15–300.75)	88.34 (56.23–120.48)	0.33	/	/
Paracetamol	n.d.	41.3 (32–50)	n.d.	No IC_{50} found by Rudnitskaya et al. [7]*	~10 mM (Rudnitskaya et al. [7])* > 7 mM (Albertini et al. [21])

Table 3
Classification proposed to predict human taste response based on rat BATA data.

IC_{50} categories	Level of aversiveness
0–0.1 mM	Extremely aversive
0.1–1 mM	Moderately aversive
1–10 mM	Mildly aversive
10–100 mM	Weakly aversive

being used as a bitter model drug there is actually very few product as bitter as it is.

4. Discussion

In this study, the taste intensity of nine model drugs was investigated at different concentrations with two different methods: the rat BATA model and a human taste panel. A strong correlation ($R^2 = 0.8963$) was found between the two *in vivo* taste assessment methods for high and medium aversive compounds. The potencies (EC_{50s}) determined in humans were essentially the same as those determined in rats suggesting that the rat BATA model is predictive of human taste assessment for these categories of compounds. For less aversive compounds, the ranking order between rats and humans slightly differs showing a lower correlation between the two taste assessment techniques. Nevertheless, the potencies determined in humans were classified in the same taste intensity categories as those determined in rats suggesting that the rat BATA model is predictive of human taste assessment for these compounds as well. It is interesting to note that despite quinine used as a bitter reference compound, very few compounds are as bitter as quinine.

Rodents possess about 30% more genes than humans [14] and probably have a wider spectrum of bitter sense detection and thus are likely to be able to detect bitter molecules with very diverse chemical structure [15]. Based on previous works undertaken with rodents, some species are more appropriate for some type of studies than others. Mice genomic map has been widely studied due to ease of genetic manipulation. Rats for their part have long been the general species of choice for behavioral studies, assessment of their behaviors are well mapped and well understood [16]. Due to their larger size, rats drink more than mice. Stellar and Hill observed that their rate of drinking is however the same, regardless of their level of thirst, and is about 1.5 mL/min. Rats drink at a rate of about 6–7 licks/s [17]; therefore it is estimated that the rats receive approximately 4 µL per lick. It is then expected to have a more consistent pattern of drinking data using rats as they have a higher capacity to drink than mice. In addition, due to their bigger size, there is an easier handling of rats compared to mice. Rats were therefore chosen to conduct the BATA experiments. Bitter taste signaling is mediated by type 2 taste receptor (T2R), a family of G protein-coupled receptors. A comparison between rat and mouse genomes led to the functional analysis of the entire rodent T2R repertoire. A total of 36 intact T2R genes and 7 pseudogenes have been found in the mouse and rat [15] compared to ~25 genes and 11 pseudogenes identified in humans [18]. They are all located on mouse chromosomes 15, 2 and 6 [19] and rat chromosomes 2, 3 and 4. Two chromosomes only comprise a single T2R gene (in rats and mice) whereas the third chromosome, chromosome 6 and 4, respectively in mouse and rat, contains the rest of the T2R genes and pseudogenes in multiple clusters, which are organized almost identically in both species. Despite some discrepancies, rodent T2R genes are highly conserved in terms of genomic organization, chromosomal localization, number of genes, sequence homology between the orthologs, tissue expression, ligand specificity and receptor function. This suggests that rodent T2Rs are evolved under similar dietary pressure and share bitter sensing functions in the lingual and gastrointestinal systems [15]. It is interesting to note that a higher number of rodent T2R genes exist compared to humans.

The EC_{50} values obtained in humans were generally within one-half log unit of molar concentration of those derived from the rat BATA experiments. These findings are in accordance with the results obtained by Devantier et al. [4] who found that the relative potencies of four drugs (quinine, ciprofloxacin, clarithromycin and nystatin) assessed in the mouse BATA assay matched the taste intensities evaluated by a trained human taste panel. In their study, the absolute potencies of the drugs in humans were also all within one-half log unit of molar concentration derived from the mouse BATA and did not differ statistically. The present results also correlate well with the findings from Rudnitskaya et al. [7] who compared the taste data obtained for eight drugs (azelastine hydrochloride, caffeine base, chlorhexidine digluconate, potassium nitrate, naratriptan hydrochloride, paracetamol, quinine hydrochloride and sumatriptan succinate) with the rat BATA model and a trained human taste panel. They found that the rat panels showed exactly the same rank order of bitterness prediction as the human panels with a consistent offset of approximately half-log unit of molar concentration.

From the results obtained in the present study, it was shown that the BATA model is reliable to predict the taste in case a human panel is not available or possible. However no general rule can be made whether rats or humans are more sensitive to the aversive taste of compounds. It has been found in this study that for quinine hydrochloride dihydrate and isoniazid, rats were more sensitive to the aversive taste than humans whereas for sildenafil citrate, ranitidine hydrochloride, diclofenac sodium, caffeine citrate, telbivudine and paracetamol, humans were more sensitive than rats. Nevertheless, the offsets were generally within one-half log unit of molar concentration except for 6-n-propylthiouracil where exactly the same potencies where found in rats and humans. Devantier et al. [4] found that mice were more sensitive than humans to quinine and clarithromycin and less sensitive to ciprofloxacin, which reinforced the present findings. However, Rudnitskaya et al. [7] found that there was an approximately consistent offset around half-log unit of molar concentration, with rats always rating the bitterness lower than humans. They explained this offset by the fact that rats were encouraged to drink as they were water-deprived whilst the human panel not. Analyzing their methodology deeper for the comparison of the rat BATA data and the human taste data, it has been noticed that the concentrations tested were not necessarily the same for both panels and that for some compounds, e.g. quinine hydrochloride, the concentrations chosen in both panels were not even overlapping. Moreover, for some of the drugs they tested, the range of concentrations chosen was lower for the human panels than for the rat panels. Therefore, this can be one of the reasons why they always found that rats were less

Table 4
Verification of the proposed classification to predict human taste response based on rat BATA data with 4 compounds [A and B: REC 4612/009; C: REC 4612/017; D: REC 4612/012].

Compound	IC_{50} (mM)	Level of aversiveness	Calculated EC_{50} (mM)	Level of aversiveness	Measured EC_{50} (mM)	Level of aversiveness
A	13.63	*Weakly aversive*	*11.28*	*Weakly aversive*	27	*Weakly aversive*
B	1.31	*Mildly aversive*	*1.34*	*Mildly aversive*	3.6	*Mildly aversive*
C	2.02	*Mildly aversive*	*1.98*	*Mildly aversive*	1.29	*Mildly aversive*
D	90.00	*Weakly aversive*	*62.87*	*Weakly aversive*	35.70	*Weakly aversive*

sensitive to the bitterness than humans.

In order to be able to have a direct comparison rats/human of taste intensity, the concentrations used in both were always overlapping. However, fewer concentrations could be tested in humans (maximum four compared to six in rats) to allow having three repeats for each concentration and two controls (positive and negative), which are essential to produce accurate results and verify the consistency of the data. Caution was also taken to minimize number of samples assessed per session to minimize taste fatigue [9,20]. Therefore, for each API, generally four out of the six concentrations assessed in the rat BATA model and covering a large range of concentrations (chosen below, at and above IC_{50} if not limited by solubility e.g. paracetamol) were selected for the human sensory analysis. It was always ensured that the concentrations chosen were well below the level causing toxicological effect in humans to make sure that in the unlikely event of an accidental ingestion of a single sample, the maximum amount of drug that participants could be exposed to would be below the acceptable daily intake (ADI). Selecting the same concentrations in both panels was essential to compare the results in an accurate manner.

5. Conclusions

The taste of nine model APIs having different degree of aversiveness (bitterness), has been assessed using two different *in vivo* methods, the rat BATA model and human taste panels. The present research showed an overall strong correlation between rats and humans taste response for the eight APIs assessed: the stronger the aversiveness, the stronger the correlation. This is an important milestone towards the use of this animal model as an *in vivo* taste assessment tool to inform the drug development including taste masking. A similar ranking order was obtained with both *in vivo* taste assessment methods for high and medium aversive compounds with a consistent offset of one half-log unit of molar concentrations between IC_{50} and EC_{50} values. However, for low aversive compounds, the ranking order between rats and humans slightly differs showing a lower correlation between the two taste assessment techniques. Nevertheless, the potencies determined in humans were classified in the same taste intensity categories as those determined in rats suggesting that the rat BATA model is predictive of human taste assessment for these compounds as well. These results indicate that the rat BATA model could be used as a taste assessment screening tool of APIs in early development. A proposed classification was made to predict human taste response based on rat BATA data.

The present new data completes the few data available in the literature comparing the taste of rodents and humans and reinforces the assertion that the rat BATA model is predictive of human taste assessment and could be used as a taste assessment screening tool of APIs in early development. In future work, additional APIs having different taste intensity levels (covering other IC_{50}/EC_{50} values) should be assessed in rats and humans to enrich the present database in order to validate the correlation found in the present study.

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