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Assessing taste without using humans: Rat brief access aversion model and electronic tongue

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There is a growing body of evidence to suggest that the organoleptic properties of a formulation (taste, smell, mouthfeel, etc.) are important determinants of patient concordance with treatment regimens (American Academy of Pediatrics, 2000; Griffith, 1990). Non concordance can have a significant effect on treatment outcomes since if a medicine is not taken it cannot exert its therapeutic effect.

The issue of unpleasant taste is particularly important for paediatric patients and for formulations where taste masking is difficult (or impossible) such as liquid dosage forms of highly soluble drug substances or those to be delivered via intra nasal or inhaled route. A survey on the burden of allergic rhinitis in American children (Meltzer et al., 2009) quotes poor taste as the second most commonly cited reason (43.7%) for patients ceasing to use their intranasal treatment for rhinitis. Similarly studies of patients using inhaled corticosteroid therapy showed that treatment compliance is often poor; poor taste affected 80% of the patients and that of all factors analysed, unpleasant taste score was most significantly different between patients of high adherence compared with medium and low adherence groups (Creer and Levstek, 1996; Harding and Modell, 1985; Milgrom et al., 1996). As well as an indirect effect on therapeutic efficacy via non compliance, there is some evidence (Shah et al., 2009) that cilia in the respiratory tract express bitterness receptors and beat faster in the presence of bitter compounds leading to faster clearance and hence a potential direct reduction in the therapeutic effect.

Generally poor taste does not become obvious until early clinical studies. If the taste is noticeable then it may unblind these studies, whilst if it is strongly aversive then it may be necessary to find a different salt of the API with better taste characteristics, or even to seek an alternative candidate. This will incur considerable delay in providing an improved treatment to patients, necessitate additional animal toxicology studies, and potentially add considerable cost to the developer.

Thus it would be very valuable to be able to screen molecules and/or salt forms early in development (preferably at the pre candidate stage) to enable the optimum molecule and/or form to be selected for further development. To undertake this in humans would require comprehensive toxicology cover, or microdosing at levels unrelated to final therapeutic doses and unlikely to yield any useful taste data. Obviously it would not be possible to perform such human studies early enough in development for them to be useful in selecting the candidate.

A number of methods have been proposed to screen the aversivness of API's and their formulations. Since these models require the compound to be in solution for testing they cannot address issues such as mouthfeel but can give valuable insights into other aspects of aversivness. These include in silico predictions (so far with limited success), in vitro methods (such as e-tongue), cell based assays (generally specific to bitterness rather than aversivness per se), isolated tongue models (limited life) and whole animal models (such as the rat brief access taste aversion [BATA] model). The presentation discusses the merits of each of these approaches concentrating on the e-tongue and rat models.

In order for the data from any of these methods to be helpful to the pharmaceutical scientist it is vital that they be predictive of the human taste response. We have undertaken a study to quantify the human, rat and e-tongue response to 9 compounds covering a wide range of chemical types and bitterness intensity. The compounds studied are presented in Table 1.

Each was assessed by a trained human sensory panel ($n = 15$ [2 males + 13 females, average age 45 years]) in a 'rinse and spit' study design at concentrations chosen to cover the expected range of bitterness from low/moderate to high at a range of molarities in quarter log steps. The panel scored the bitterness on an anchored 7 point scale. The bitterness was then converted to a % bitterness score. Samples were assessed during 9 sensory sessions. A single 'calibration' concentration of quinine was used in each session to ensure that data remained consistent between sessions.

The same compounds were also assessed using the BATA model and a new electronic tongue that is currently under development.

Table 1
Compounds studied and their use.

Compound	Use
Quinine HCl[a]	Anti malarial – bitterness standard
Chlorhexidine di gluconate	Antibacterial – mouthwash
Azelastine HCl	Rhinitis
Naratriptan HCl	Migraine headaches
Sumatriptan succinate	Migraine headaches
TegoBetaine	Surfactant – toothpaste ingredient
Caffeine	Stimulant – bitterness standard
Paracetamol	Analgesic/antipyretic
Potassium nitrate	Toothpaste ingredient

[a] A single concentration of 2.0×10^{-3} g/l defined as 'moderate bitterness' was used as a control to calibrate the panelist's responses in all studies.

Table 2
Results of the e-tongue bitterness intensity prediction against known human data.

Substance	Mean relative error (MRE) (%)
Azelastine	24
Caffeine	17
Chlorhexidine	5
Potassium Nitrate	11
Naratriptan	19
Paracetamol	17
Quinine	2
Sumatriptan	34

MRE is the averaged absolute deviation of the predicted value from the measured one for the range of concentrations tested.

In the rat brief access aversion model rats are offered access to various drinking bottles in a controlled sequence for a controlled time and the number of licks that they take is measured electronically. The bottles presented contain water, a calibration compound at a fixed concentration or the compound under test at various concentrations. If the rat finds the sample presented to be aversive it will lick less often relative to the water sample. A % lick inhibition can then be calculated. The same rat is presented with the bottles in randomised order a number of times in a single experiment. Thousands of data points can readily be generated, using very few animals (generally 6), leading to a robust aversion concentration curve. Observations and animal husbandry data confirm that the rats do not find this experimental procedure stressful. "All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals".

For the electronic tongue (Legin et al., 2004, 2011) the potentiometric response pattern of a range ($n = 27–30$) of semi selective electrodes placed into solutions of the same compounds as above at a range of concentrations and several pH's was measured. The measurements were repeated a number of times and the data were merged into a three-dimensional data set (samples × sensors × pH) and calibration models were calculated using 3-way PLS regression versus the human data or the rat data. The model was internally validated by the 'leave one out' method (excellent correlation and MRE around 7%) and the predicted bitterness of each compound was calculated from the model as if it were an unknown. The standard error of prediction calculated for each compound confirmed the predictions to be reasonably good. A simplified data set is shown in Table 2.

Validation studies are ongoing, testing the model with additional compounds where the in vivo sensory data has not been revealed to the e-tongue team (Fig. 1).

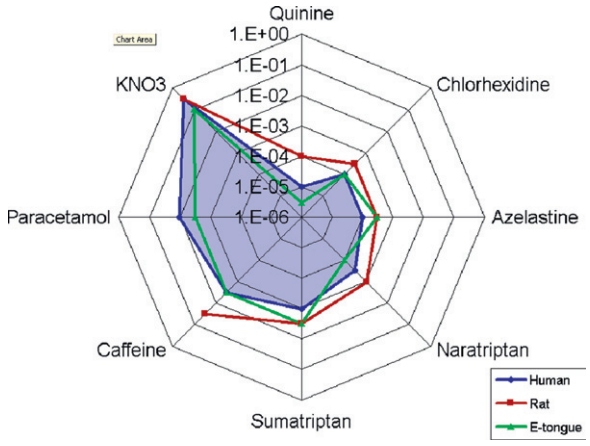

Fig. 1. Comparison of human, rat and e-tongue data ED_{50} values.

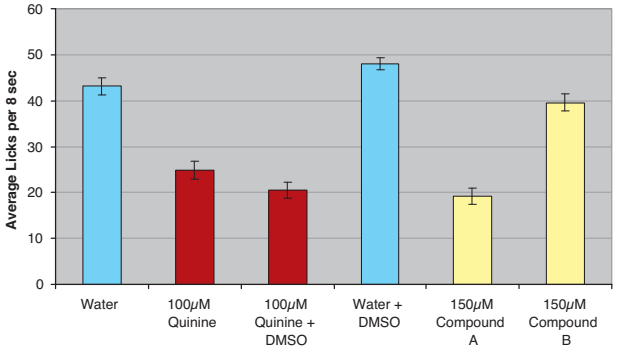

Fig. 2. An example application of the BATA.

To simplify comparison between the three methods an ED_{50} (the concentration that produced or predicted a 50% aversion score) was calculated. It can be seen that the agreement between models is excellent. The lack of an ED_{50} value for paracetamol in the rat is explained by the poor solubility of the compound, leading to a flat dose response.

The generally good overlap between the e-tongue and human data is not too surprising since the PLS regression model was developed using the human data. Close inspection shows that although prediction is generally good, the e-tongue would occasionally predict some slight rank order differences. The model would be expected to improve as further data is added. The rat data shows exactly the same rank order of bitterness prediction as the human panel, with an approximately consistent offset of ca ½ log unit of molar concentration. This offset appears to be highly predictive and can be explained by the fact that the rats are encouraged to drink whilst the human panel is not.

Having established the predictive nature of the models, particularly in the rat, the data can be used to test compounds of interest. An example is reported in Fig. 2.

This plot confirms that compounds can be differentiated from each other and that Compound B, being significantly less aversive than Compound A, would be expected to be easier to develop. It also helps us to understand the likely patient response since the taste can be compared to a standard, such as quinine, which has well recognised taste characteristics and which has been assessed by the human taste panel.

The aim is to further develop these methods to allow them to be used routinely as part of the candidate selection/version selection decision for new API's. Provided that ongoing validation of the e-tongue continues to support its utility we anticipate that we will use this instrument to screen molecules during the discovery phase. When a fully physiological response is required to distinguish between pre candidates we intend to use the rat model. As more data on the accuracy of prediction from the e-tongue becomes available, and more data is added to the model, it may be possible to entirely replace the BATA.

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Industry perspective on palatability testing in children—Two case studies

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1. Introduction

The development of age appropriate paediatric formulations is paramount to enable children adherence to treatment (Mennella and Beauchamp, 2008). It encompasses multi-dimensional considerations including the administration route, the formulation technology and the dosage strength. For oral treatments, palatability is crucial for children compliance to therapeutic regimens (Matsui et al., 1997). EMA Paediatric Investigation Plan (PIP) guidelines stress the particular relevance of taste masking and palatability testing in the development of oral treatment for children (EMA, 2007). The need for taste testing of new medicines was also recognized by the French Health Products Safety Agency, AFSSAPS, who is proposing paediatric taste acceptability studies for liquid antibiotic preparations (Cohen et al., 2009).

Therefore, taste masking and taste testing is becoming intrinsic to paediatric pharmaceutical development. The taste information can be derived from several methods like the electronic tongue, cell and animal based models and human panel testing (Anand et al., 2007). The taste testing in children is considered as the most reliable approach as the taste prediction by in vitro methods still lacks understanding while taste perception and preferences have been shown to be different from adults to children (Matsui et al., 1997).

However, carrying out taste tests in children is associated with a variety of practical, technical, ethical and regulatory challenges, including enrolment of children, lack of regulatory guideline, questionnaire design and reliability of paediatric responses (Cram et al., 2009).

General practical considerations related to palatability testing in children, e.g. questionnaire and response model design will be discussed. These will further be exemplified with 2 case studies.

1.1. Palatability studies

Palatability assessment typically comprises the taste assessment, e.g. the measure of the taste quality and intensity to encompass initial taste, aftertaste, flavour and texture. For this assessment, several types of questionnaire are used, including various types of response options. The mostly used response options types are (van Laerhoven et al., 2004):

- The verbal categorical response option is based on scoring of taste in a scale of e.g. 1 (very good) to 5 (very bad)
- The pictorial categorical response option (using a facial hedonic scale) allows expression of preferences using a pictorial scale
- Modified numeric response option is a combined visual analogue scale (VAS) and facial hedonic scale (Fig. 1)

Cognitive capabilities of the child have to be reflected in the design of the questionnaire and the choice of the response options model to ensure a reliable study assessment.

From 4 years onwards, children can generally well communicate their feelings and preferences and are therefore considered capable of participating in taste assessment trials (Sjovall et al., 1984). However, for children below 6 years, it is not recommended to use the facial hedonic scale alone as these young children may associate the facial pictures with other quality attributes (or their own mood) than the taste. In addition, they may not be able to express differences in taste perception and rank formulations. Therefore for children below 6 years it is recommended to use the child's own spontaneous verbal judgment following a control question when comparing different formulations (Anand et al., 2008). In order to improve the reliability of the study outcome, parents, caregivers and/or health providers should be involved in the study and asked to report about any discomfort or other observations in relation to acceptance of the study medication (e.g. spitting out of the medicine).

For children younger than 4 years of age neither the spontaneous verbal judgment nor the facial hedonic scale can be used as they have limited ability to communicate, understand the questionnaire and follow the study instructions. Hence, the questionnaire is designed and limited to collect the observations and their interpretation from parents, caregivers and/or health providers only. Therefore alternative response models can be used like the facial coding system for pain quantification or the use of behavioural elements of the medication acceptance scale, e.g. cry, facial expression or body movement (Kraus et al., 2001).

Two case studies (at a late stage and an early stage of pharmaceutical development) of palatability assessment in children are discussed in the following paragraphs. More detailed information regarding the methods and results can be accessed in the original papers Abdulla et al. (2010) and Saez-Llorens et al. (2009), writ-

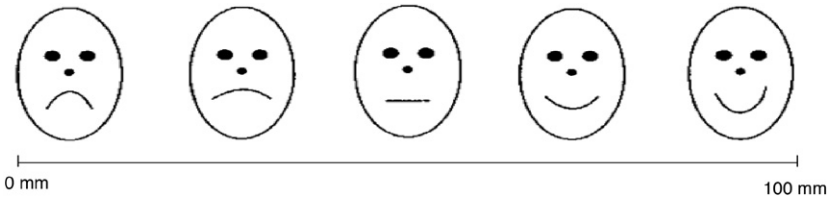

Fig. 1. Modified visual analog scale including a 5-point hedonic scale often used to assess the palatability of paediatric formulation.