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Improved smell function with increased nasal mucus sonic hedgehog in hyposmic patients after treatment with oral theophylline

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ABSTRACT

Purpose: We previously demonstrated the presence of sonic hedgehog (Shh) in nasal mucus in normal subjects and in patients with smell loss (hyposmia). Nasal mucus Shh levels were found significantly diminished in untreated hyposmic patients of multiple etiologies. Since treatment with oral theophylline has been previously associated with improvement in smell function we wished to study if such treatment increased nasal mucus Shh as well as improved smell function in patients with hyposmia.
Methods: Forty-four patients with hyposmia of several etiologies were evaluated for changes in hyposmia by subjective measurements of smell, taste and flavor perception and by olfactometry. Measurements of nasal mucus Shh were made in relationship to each set of sensory measurements. Patients were treated with oral theophylline at doses of 200–800 mg for periods of 2–10 months with sensory function, nasal mucus Shh and serum theophylline levels evaluated at these time intervals. Nasal mucus Shh measurements were made with a sensitive spectrophotometric ELISA assay and theophylline with a fluorometric assay.
Results: There was consistent, significant improvement in subjective responses in smell, taste and flavor perception and in olfactometry associated with increased nasal mucus Shh and serum theophylline after theophylline treatment.
Conclusions: Improvement in smell function and in nasal mucus Shh was positively correlated in a dose-response relationship after treatment with oral theophylline. Results are consistent with a successful role for theophylline in improvement of smell function in hyposmic patients of multiple etiologies associated with increased nasal mucus Shh which can act as a biochemical marker for smell function.

1. Introduction

We previously demonstrated the presence of sonic hedgehog (Shh) in nasal mucus in normal subjects and in patients with loss of smell (hyposmia) related to several etiologies [1]. We have also demonstrated that nasal mucus Shh levels in these untreated hyposmic patients were significantly lower than in normal subjects [1]. Since prior studies demonstrated that treatment with oral theophylline increased smell function in hyposmic patients [2,3] we now wish to evaluate whether theophylline treatment increased nasal mucus Shh as well as improved smell function in hyposmic patients.

Thus, the purpose of the present study is to present results of changes in nasal mucus Shh in relationship to changes in smell, taste and flavor perception and in olfactometry in relationship to Shh levels in nasal mucus and in serum theophylline levels before and after oral theophylline treatment in patients with hyposmia.

2. Methods

2.1. Subjects

2.1.1. Normal subjects

Fourteen volunteers aged 22–84 y, 62 ± 4 y (Mean ± SEM), six men, eight women with normal smell and taste function were studied. These subjects were either patients who presented to The Taste and Smell Clinic in Washington, DC for evaluation of symptoms unrelated to smell loss or who were employees of The Taste and Smell Clinic who volunteered for the study. Subjects included all those who volunteered for the study.

2.1.2. Patients

Forty-four patients aged 10–88 y, 56 ± 3 y who presented to The Taste and Smell Clinic in Washington, DC for evaluation and treatment of smell loss were also subjects of the study. Patients were treated with oral theophylline at The Clinic from 2012 to 2013 with a dose

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range of 200–800 mg taken over a period of 2–10 months. Changes in smell function and in nasal mucus Shh were made at these intervals of 2–10 months after theophylline treatment.

All patients reported smell, taste and flavor loss by subjective statement as previously described [2,3]. Smell loss was measured by olfactometry [2,3], as previously described. Olfactometry consisted of determination of detection (DT) and recognition (RT) thresholds, magnitude estimation (ME) and hedonic evaluation (H) for four odors (pyridine, nitrobenzene, thiophene and amyl acetate) [2,3]. Olfactometry abnormalities in untreated patients consisted of increased DT and/or RT above normal (decreased sensitivity) and/or decreased ME (decreased sensitivity) for one or more of the odors and/or decreased unpleasantness for odors of pyridine and thiophene or variable changes in pleasantness for odors of nitrobenzene or amyl acetate [2,3].

Patients exhibited six etiologies related to their smell loss: post-influenza-like hyposmia (PIHH) [4] (10 patients), allergic rhinitis [5] (15 patients), congenital loss of smell [6,7] (nine patients), head injury [8] (eight patients), post general anesthesia [9] (one patient) and dysgeusia (distorted taste sensations) [2,3] and oropyrosis [10] (one patient).

Improvement in smell function after oral theophylline therapy consisted of improvement in both subjective sensory perceptions and in olfactometry [2,3]. Subjective improvement in smell, taste and flavor consisted of improvement in perception for all odors, tastes (salt, sweet, sour and bitter) and flavor (for all foods and beverages) based upon a 0–100 scale with 100 indicating complete recovery of smell perception with responses from 0 to 100 scaled between 0 and 100 (as previously described) [3]. Improvement in flavor perception (i.e., the flavor obtained from the taste of foods and beverages) was also measured by responses on a 0–100 scale with 100 indicating that all flavors of food and beverages were considered normal and responses < 100 scaled consistently less [3]. Subjective improvement in taste function was measured with changes in taste for salt, sweet, sour and bitter tastants evaluated on the same 0–100 scale with 100 indicating return to normal for each of the four tastants and responses < 100 scaled consistently less [3]. Improvement by olfactometry was indicated by decreased DTs or RTs (increased sensitivity), increased MEs (increased sensitivity) and changes in H consistent with increased sensory perceptions for usually considered unpleasant odors (more unpleasant) or increased hedonic pleasantness for odors usually considered pleasant (more pleasant) [3].

Olfactometry improvement reflects specific changes in sensory function. Decreased DTs after theophylline treatment reflect increased olfactory detection with increased receptor growth and sensitivity [3]. Decreased RTs after this treatment reflect increased sensitivity in olfactory receptor-brain relationships [3]. Increased MEs reflect increased olfactory receptor number [3]. Changes in H reflect changes in brain and receptor function related to changes in olfactory perception [3].

Results were analyzed by several techniques. Comparison of subjective responses between improved and non-improved responses to theophylline treatment were performed using X^2 with $p < 0.05$ considered significant. In addition mean ± SEM of subjective responses in each category of improvement or lack of improvement were also obtained by use of arithmetic means. Mean ± SEM levels in each category of olfactometry function were obtained and results compared both before and after theophylline treatment using Student *t*-tests with $p < 0.05$ considered significant. Pearson product-moment correlations were obtained related to theophylline dose and nasal mucus Shh levels with $p < 0.05$ considered significant. Analysis of variance (ANOVA) among theophylline dose, nasal mucus Shh levels and smell function were also obtained with $p < 0.05$ considered significant.

Study protocol was approved by the Chesapeake Institute Review Board. Each patient and subject agreed to participate in the study and signed an informed consent participation form.

3. Methods

Patients and volunteers collected all nasal mucus they spontaneously produced over a period of 1–4 days into a 50 ml plastic tube. All samples were refrigerated overnight for collections longer than 24 h.

Each sample was transferred to a 12 ml plastic tube and centrifuged in a refrigerated RC5C Plus Sorvall centrifuge at 18,000 rpm for 45–55 min. Supernatant was transferred to PCR tubes and stored at −20 °C until analyzed.

Each nasal mucus sample was analyzed by use of a sensitive spectrophotometric ELISA technique obtained from Abcam Inc. (Cambridge, MA). Analysis of duplicate samples agreed within 5%. All analyses were made independent of the knowledge of the status of any subject. Only after all samples were analyzed were results tabulated and samples classified in relationship to subject status.

Serum theophylline was measured by a standard fluorescence assay [11].

4. Results

4.1. Subjective sensory changes

Subjective improvement in smell function following treatment with oral theophylline is shown in Table 1. Of the patients treated with oral theophylline 61% reported improvement with a range of 2–100% and a mean improvement of 34 ± 6%. Of these improved patients 58% were men with an improvement of 27 ± 9% and 42% were women with an improvement of 43 ± 17%.

Flavor perception was also reported improved in 61% of the patients consistent with their stated improvement in smell function. Taste function was reported improved in 64% of the patients consistent with their improvement in smell and flavor perception.

4.2. Olfactometry changes

Smell function in hyposmic patients as measured by olfactometry before and after oral theophylline associated with levels of Shh in nasal mucus is shown in Table 2. Smell function in the untreated hyposmic patients were significantly impaired with respect to normal subjects as measured by increased DTs and RTs (decreased sensitivity) for pyridine, thiophene and amyl acetate and increased DT (decreased sensitivity) for nitrobenzene. After treatment with oral theophylline there was significant improvement in smell function in several but not all categories (Table 2). There were significant decreases (increased sensitivity) with respect to the untreated state in DTs for pyridine, nitrobenzene, thiophene and amyl acetate and RTs for nitrobenzene. MEs for all odors increased (increased sensitivity) except for thiophene. Hs

Table 1
Subjective responses to oral theophylline treatment in hyposmic patients treated with oral theophylline.

Condition	Number studied [%]	Response range [%]	Response Mean ± SEM
Smell function (31)			
Improved*	19 [61]	2–100	34 ± 6
Men	11	3–95	27 ± 9
Women	8	2–100	43 ± 17
Not improved	12 [39]	0	0
Flavor function (31)			
Improved*	19 [61]	1–100	43 ± 8
Not improved	12 [39]	0	0
Taste function (31)			
Improved**	20 [64]	3–100	47 ± 8
Not improved	11 [36]	0	0

( ) Patient number. [ ] % of patients studied. With respect to not improved.
* $p < 0.05$, X^2.
** $p < 0.01$, X^2.

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