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Short Communication

Use of the Local Anesthetic Lidocaine to Reduce Pain during Nasal Brushings

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Abstract

Airway epithelial cells are the first to be exposed to air-borne stressors, and are crucial for the initiation and regulation of immune responses. The *ex vivo* cultivation of primary nasal epithelial cells (NECs) closely mimics the human *in vivo* situation, and assists us in the study of their role *in vitro*. NECs can be obtained via superficial brushes using an easy to learn technique, and are more easily accessible compared with bronchial epithelial cells; however, performing nasal brushings in order to obtain NECs from volunteers can be painful.

Therefore, we tested the effect of the local anesthetic lidocaine to reduce pain during nasal brushings in an un-blinded, controlled, cross-over study in 13 healthy adolescent volunteers. We randomly assigned one nostril to be anesthetized, and brushed both nostrils. Outcome measures were cell yield and growth, as well as pain perception using a standardized score. Four weeks later, the procedures were repeated while the other nostril was anesthetized.

The use of lidocaine did not affect cell yield or cell growth, while pain was significantly reduced. The median pain was reduced by 1.5 score points, from 3.5 (25-percentile = 2; 75-percentile = 4.25) for the reference nostril to 2 (1; 3) for the lidocaine-treated nostril (p=0.009).

In conclusion, the use of lidocaine for nasal brushings resulted in less pain for the subjects, while cell yield and viability remained unaffected. Its use can thus be considered in those subjects that would otherwise decline to participate in such studies.

Keywords: Nasal Epithelial Cells; Airway Epithelial Cells; Bronchial Epithelial Cells; Airways; Respiratory Epithelium

Introduction

Airway epithelial cells (AECs) are one of the first sites within the human body to be exposed directly to inhaled environmental stressors. Epithelial cells play a crucial role during the initiation and regulation of immune responses [1-3], acting as a barrier, and providing a source of cytokines and chemokines. Further, AECs are involved in various pulmonary diseases, such as bacterial and viral infections, cystic fibrosis, asthma, or chronic obstructive pulmonary disease.

All of these factors together make AECs an interesting bio-

logical target. The use of AEC lines, however, or AECs from animals does not adequately reflect the human *in vivo* situation. Therefore, using human primary nasal epithelial cells (NECs) offers an ideal *in vitro* model, closely mimicking the human *in vivo* situation. While it is highly invasive to obtain bronchial epithelial cells (BECs), NECs can be obtained by performing superficial brushes without systemic anesthesia. The brushing technique is easy to learn, volunteers can be brushed repeatedly, and the procedure can be conducted in diseased populations, which would otherwise not qualify for research-related bronchoscopies.

Even though the acquisition of NECs via nasal brushings is less invasive than a bronchoscopy, the brushing can be associated with pain. The use of local anesthetics, such as lidocaine, is thought to make nasal brushings less painful [4]. However, the effects of lidocaine on cell yield and growth were unknown. The aim of our research, therefore, was to study the effect of lidocaine on the yield and growth of NECs, as well as on pain during the nasal brushing.

Material and Methods

Study Design and Subjects

We conducted an un-blinded, controlled, cross-over study with 13 volunteers (mean age = 17.7±0.48 (standard deviation) (range = 17-18); 100% male subejcts; mean body mass index = 20.5±1.6 (range = 17.1-22.6); all subjects were healthy, no diseases affecting pain perception or airways, one was treated for acne) in order to test the effects of lidocaine on cell yield and growth, and on pain perception during nasal brushings. Exclusion criteria included smoking, bleeding disorders, use of anticoagulants, respiratory infections, and asthma. The study was approved by the Ethics Committee of Basel, Switzerland. Written informed consent was obtained for all participants.

Initially, the authors applied 1mL lidocaine via syringe into one nostril of the volunteers while their head was inclined. After seven subjects had completed the study, we received multiple complaints about the unpleasant side-effects, such as an anesthetized throat, from swallowing the lidocaine. As a result, for the second group, we changed the route of application to self-administration by the subjects via a nasal spray. The second study group (N=7, one volunteer was in both groups) applied the lidocaine themselves via five puffs from a conventional nasal spray. In this paper, we will separately report the results for both the first (syringe) and second groups (nasal spray).

We randomly assigned one of the nostrils to be anesthetized using lidocaine 10mg/ml (Rapidocain® 1%, Sintetica®), and then brushed both nostrils. In order to allow the lidocaine to take effect, the non-anesthetized nostril, and then the anesthetized nostril, was brushed (or at the earliest, one minute after lidocaine application). Each subject came back for a second brushing (at least four weeks after the first one), at which point the opposite nostril was anesthetized.

Nasal Brushings

Subjects underwent two brushings of both nostrils using interdental brushes (method see [5, 6]). Briefly, interdental brushes were fixed to pipette tips with parafilm and wetted with phosphate buffered saline (PBS; Sigma-Aldrich, Buchs, Switzerland). The brushes were inserted into the nostril, moved in a bidirectional and rotational manner, and stored in PBS until processing. Two brushings per nostril were used and pooled into one 15ml tube, resulting in one sample per side.

Cell Yield and Cell Growth

Cells were processed as previously described [5, 6]. Briefly, after vortexing, the brushes were moved up and down in PBS through a cropped pipette tip. Tubes were centrifuged (300rcf, 5min) and the cell pellet re-suspended in 1ml bronchial epithelial growth media (Lonza; Ruwag Lifescience, Bettlach, Switzerland). Brushes from the two nostrils were processed separately, and cells were counted blinded using trypan blue (Sigma-Aldrich) staining for viability assessment (cell yield endpoint). Cells were seeded in a 12.5cm^2 tissue culture flask (BD Falcon; Milian, Nesselnbach, Switzerland) pre-coated with bovine collagen (PureCol; Advanced Biomatrix, San Diego, USA) and stored at 37°C, 5% CO_2, and 80% humidity. Usually one week after seeding, after reaching 80% confluence, cells were lifted and transferred to 25cm^2 tissue culture flasks. Another week later, at 80% confluence, cells were counted again (cell growth endpoint).

Pain Perception and Side Effects

Pain perception and side-effects were evaluated via questionnaire focusing on the difference between both nostrils (with and without lidocaine), including a rating-scale for pain quantification (0-10, based on www.schmerzskala.de/schmerzskala.html). The numbers represent: 0=no pain, 1=very mild pain (tickling), 2=mild pain (scratching), 3=persistent mild pain (uncomfortable, no need to stop), 4=moderate pain (very uncomfortable, need to stop), 5=moderate-serious pain, 6=serious pain, 7=serious-severe pain, 8=severe pain, 9=very severe pain and 10=worst pain possible.

Statistics

Data are presented as median and 25- and 75-percentiles. Statistical analysis was performed using paired t test. $P<0.05$ was considered as statistically significant.

Results

All subjects completed the study and all brushings were performed successfully. Cells of six volunteers had to be disposed of (due to fungal contamination), and from one subject, the cell yield was too low to be further analyzed.

Syringe Part

Lidocaine application by syringe had no significant effect on the yield of living cells, (Figure 1A) nor on the viability rate

(23% (15; 43) for reference, 23% (9; 44) for anesthetized nostrils). The median cell yield of the syringe group was $1.9x10^5$ living cells (25-percentile=$0.5x10^5$; 75-percentile=$4.6x10^5$) without lidocaine and $1.0x10^5$ living cells ($0.3x10^5$; $3.1x10^5$) with lidocaine.

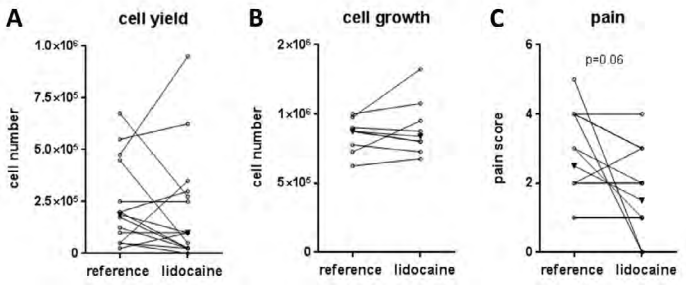

Figure 1. Cell yield, cell growth and pain perception in the syringe study group. (A) Cell number after the brushing was assessed for each nostril individually. **(B)** Cell number after cultivation for approximately two weeks (assessed for each nostril separately). **(C)** Pain scored by the subjects for each nostril separately. Triangles represent the median values of the groups. Differences tested with paired t test.

Cell number after reaching confluence in the second tissue culture flask was not affected by lidocaine treatment (Figure 1B). Reference nostrils yielded $8.8x10^5$ cells ($7.4x10^5$; $9.6x10^5$), the anesthetized nostrils $8.4x10^5$ cells ($7.4x10^5$; $10.4x10^5$).

Lidocaine reduced pain for the volunteers, albeit only with borderline significance and in a heterogeneous manner (Figure 1C). The median value of pain perception dropped from 2.5 score points (1; 4) to 1.5 (1; 3) (p=0.06). After application of lidocaine via syringe, 29% of the volunteers reported an anesthetized throat, and 57% reported a runny nose and watery eyes (Table 1). Runny nose was more frequent in the lidocaine-treated subjects, as compared to reference nostrils.

Table 1. Side effects of the nasal brushings reported by the subjects.

	Syringe study group (N=7)		Spray study group (N=7)	
Side effect	**reference**	**lidocaine**	**reference**	**lidocaine**
Runny nose	36%	57%	43%	43%
Watery eyes	57%	57%	64%	64%
Anesthetized throat	-	29%	-	0%

Spray Part

Lidocaine application via spray did not affect the yield of living cells (Figure 2A), nor the viability rate (21% (14; 35) for samples without and 20% (12; 34) for samples with lidocaine). The median cell yield was $1.3x10^5$ living cells ($0.5x10^5$; $2.1x10^5$) without and $1.6x10^5$ living cells ($0.4x10^5$; $2.3x10^5$) with lidocaine.

After reaching confluence in the second tissue culture flask, the cell numbers were not different between the treatment groups (Figure 2B): We yielded $10.1x10^5$ cells ($8.1x10^5$; $13x10^5$) for the reference and $8.4x10^5$ cells ($7.8x10^5$; $12.2x10^5$) for the lidocaine-treated nostrils.

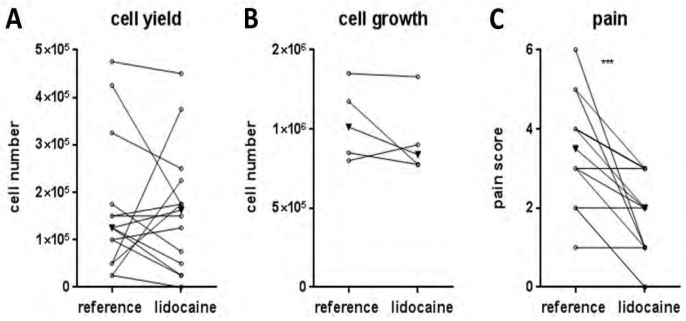

Figure 2. Cell yield, cell growth and pain perception in the spray study group. (A) Absolute number of living cells after the brushing (assessed for each nostril individually). **(B)** Cell number after cultivation for approximately two weeks. **(C)** Pain scored by the subjects for each nostril separately. Triangles represent the median values of the groups. ***p<0.001, significantly different if tested with paired t test.

Treatment with lidocaine significantly reduced pain for the volunteers (Figure 2C). While the median value of pain perception was 3.5 (2; 4.25) in reference nostrils, it was only 2 (1; 3) (p=0.0009) in lidocaine-treated nostrils. Side effects (runny nose and watery eyes) were reported as often for reference as for anesthetized nostrils (Table 1).

Discussion

Our median cell yield range was between $1.0x10^5$ and $1.9x10^5$ living cells, which is in the range of Hussain et al. (2014) (average of $1.57x10^5$ living cells) [4]. The viability of the cells in their study was 40-50%, and thus higher than ours (20-23%). One reason may be due to the nose washing they performed before the brushings, which may remove dead cells and thus increase viability, but not the absolute number of living cells. We found no effect on cell growth and thus no limitations for lidocaine use.

We also showed that lidocaine application by spray reduced the pain while having no significant side effects. Hussain and colleagues [4] did not report any side effects in their study. However, they did not include a non-anesthetized control, nor was pain assessed. Lidocaine application was also slightly different than in our study: Lidocaine was "sprayed on the nasal mucosa by gently holding the nostrils wide open with a sterilized rhinoscope". Additionally, they used lidocaine at a higher concentration (34mg/ml versus 10mg/ml). Their lidocaine also contained naphazoline, which acts rapidly as a vasoconstrictor, reducing swelling of the mucous membranes, which could potentially reduce undesirable side-effects.

NECs are a great model of the human airways for mimicking the *in vivo* situation. Several studies have shown that NECs of-

ten behave similar to BECs, and are suitable as a surrogate for BECs [7-9]. This is a big advantage given that obtaining NECs is less invasive, and primary NECs are an ideal model of the respiratory mucosa.

We included a non-anesthetized comparison in our study, and controlled for anatomical differences between the two nostrils. However, we only included young, male subjects, whose response to the lidocaine and nasal brushings might differ from female subjects, or older people. Since the effects of local anesthetics were obvious for the volunteers, we did not include a placebo control. The main conclusion of our study, however, was not affected by these limitations.

Conclusion

We conclude that the use of lidocaine as a local anesthetic during nasal brushings does not affect cell yield or growth, but does significantly reduce pain for the volunteers. We recommend using lidocaine via spray application as a means to reduce pain and increase the tolerability of nasal brushings in those subjects who would otherwise decline to participate in studies of this kind.

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