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Research paper

In vitro evaluation of a protective nasal spray: Measurements of mucoadhesion and reconstructive barrier properties towards a tracheobronchial reconstruct

Giuseppina Sandri, Francesca Saporito, Franca Ferrari, Maria Cristina Bonferoni, Silvia Rossi, Carla Caramella*

Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

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ABSTRACT

Upper respiratory tract infections represent a common acute illness. Inflammation due to local infections could be managed by mucosal protection due to mechanical barrier. This strategy represents an innovative approach other than pharmacological treatment, which may concur to reduced antibiotic use.

A disrupted barrier could be restored by application of substances interacting with the mucus layer that covers the mucosa and exert intrinsic barrier properties.

Aim of the present paper was to evaluate the restoration of a barrier disrupted by inflammation, by means of a medical device designed for nasal application (nasal spray, NS formulation). The NS components were characterized by means of metabolomic analysis. NS formulation was characterized for mucoadhesion by an inclined plane method. Its barrier properties were assayed towards inflamed EpiAirway™ (MatTek Corporation, Ashland, USA), a 3D organotypic substrate exposed to interleukin 13 (IL-13), to induce goblet cell inflammation, a condition common in the pathogenesis of upper airway diseases. NS formulation showed good mucoadhesion and biocompatibility towards EpiAirway. It was able to restore epithelial integrity and the native and physiological barrier properties. Histological analysis confirmed the capability of NS formulation to reestablish barrier properties of cell substrates.

1. Introduction

Upper respiratory tract infections represent the most common acute illness in the community. They can range from the self-limiting common cold, to viral and bacterial infections of pharynx and larynx and to inflammation caused by irritating agents. Respiratory viruses, bacteria and irritants cause a widespread inflammation of the mucosa of several anatomical areas, especially the upper respiratory tract mucosa.

Static and dynamic mechanisms (structure of the epithelium, configuration of endonasal airflow) and regulated physical and chemical mechanisms (structure and content of nasal mucus, mucociliary clearance, nasal cycle, plasma extravasation by NO) assist in immune defense. Epithelial cells have a key position as a physical barrier and are the mainly responsible cells for maintaining the mucociliary transport [1]. Starting from the above assumptions, it seems reasonable that the protective effect exerted by a mechanical barrier may represent a reliable therapeutic approach and should effectively limit the epithelial damage caused by microorganisms and irritants on the mucosa. Such a strategy represents an innovative therapeutic approach other than pharmacological treatment which has been previously demonstrated to have an antitussive effect in contact with the pharynx mucosa [2].

The barrier effect is achieved by a particular combination of selected specific fractions obtained from natural sources such as resins and polysaccharides. Resins are sticky, water-insoluble substances often exuded by certain plants in response to a damage, probably as a defense mechanism. Their composition mainly comprises diterpenes, but also alcohols, phenols or gums. Resins have astringent properties; astringency affords increased protection to the subadjacent layers of the mucosa from micro-organisms and irritant chemicals. Polysaccharides, thanks to their large, highly branched polymeric structure, have good bioadhesion properties and are able to absorb water and swell to many times their original volume. As a consequence of these properties, the mucosa is bound

* Corresponding author.
E-mail address: carla.caramella@unipv.it (C. Caramella).

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tightly and rendered less permeable [3].

The restoration of mucosal integrity is hypothesized to be due to the formation of a protective barrier that prevents continuous contact with external irritants. Such a barrier interacts with the mucus layer, which covers the mucosa, maintaining a protective coating for prolonged time period to ensure an effective insulation towards substances involved in local infection.

In this perspective, the investigation of the performance of a mechanical therapeutic intervention should clarify the impact of this approach in the management of upper airway disease.

The aim of the present paper was to evaluate the barrier effect of a medical device designed for nasal application (nasal spray, NS formulation) containing resins and polysaccharides as main functional components. The components were characterized by means of metabolomic analysis. Primarily NS formulation was characterized for its mucoadhesive properties by means of the inclined plane method, subsequently its barrier restoration properties were assayed towards inflamed EpiAirway™ (MatTek Corporation, Ashland, USA), a 3D organotypic culture that consists of normal, human-derived tracheal/bronchial epithelial cells which have been cultured to form a pseudo-stratified, ciliated, columnar epithelium. This 3D model reflects normal human bronchiole histology and forms differentiated pseudo-stratified cell layers resembling the epithelial tissue of the respiratory tract, including nasal epithelium. It possesses microvilli and cilia on the apical surface of the cultures and the cells are capable of mucin secretion. Moreover tight junctions are present between the cells [4].

According to standard protocol, EpiAirway™ substrates were exposed to interleukin 13 (IL-13) to induce inflammation of the epithelium, a condition common in the pathogenesis of upper airway diseases [5], subsequently, the permeation of lucifer yellow and the evaluation of TEER (transepithelial electrical resistance) were measured, as markers of mucosal integrity, in presence and without NS, in order to evaluate the capability of the device to adhere to the tissue and restore its impaired barrier.

The quality of NS formulation was controlled by means of metabolomic fingerprint ESI-MS analysis, a fast and effective analytical method able to give a fingerprinting of the product, on the basis of the components declared [6].

2. Experimental part

2.1. Materials

The formulation tested is a nasal spray (NS), a medical device marketed in Italy as Rinosol 2Act® and containing: Aloe Vera gel dry extract; Hamamelis leaves freeze dried extract; Green tea leaves freeze dried extract; Myrrh freeze dried extract; Eucalyptus essential oil; Niaouli essential oil; Xanthan gum; Benzylic alcohol; Potassium sorbate; vegetable oils; Glycerin, Lauryl glucoside, Polyglyceryl-2-Dipolyhydroxystearate, Glyceryl Oleate, Dicaprylyl Carbonate.

2.2. Methods

2.2.1. Product characterization: metabolomic analysis

In this investigation we studied one batch of NS formulation to observe the behavior when stored at different experimental condition (25 °C ± 2°C/60%RH°±°5%RH, 40 °C ± 2°C/75%RH°±°5%RH) for different months.

The product stability was studied by means of Metabolomic ESI-MS fingerprint analysis [7]. 0.5 g of each sample was suspended in 25 ml of ultrapure water and diluted 1:2 with methanol. The solutions were directly infused in the ESI source of an Agilent 1100 Series LC/MSD ion trap by FIA method with flow rate of 0.2 ml/min and an injector volume of 5 μl. The composition of the mobile phase was: A) pure water, and B) Methanol for LC-MS (grade of purity 99.9%), (Sigma–Aldrich, Milan).

The instrumental parameters used for ESI analysis are reported in Table 1.

Due to the high complexity of the metabolic profile of the product under investigation, the mass spectral data were evaluated by multivariate and monovariate analysis.

The alignment of the spectra was obtained using the software R (Version 3.0.2, the R Foundation for Statistical Computing). The revised data were saved in CSV format and the matrix in CSV format has been reworked by the program SIMCAP+ (Version 13.0.0.0, 28 March 2012).

2.2.2. Mucoadhesion measurements by means of "inclined plane"

Mucoadhesion measurements were performed by using "inclined plane" apparatus. It basically consisted of an inclined plane (angle of inclination = 45°, surface area = 28 cm^2) thermoset at 37 °C and of an electronic microbalance (MS303SE, Mettler Toledo, Novate Milanese (MI), I) connected with a personal computer.

Porcine gastric mucin (type II) (Sigma, Italy) was used as biological substrate.

Mucin films were prepared directly on the plane: 2.5 ml of a 8% w/w mucin dispersion in water were placed on the plane, which was kept horizontal at 45 °C for 45 min to dry [8,9].

1 g of formulation was placed on the plane previously coated with the biological substrate and kept horizontal.

Then the plane was inclined and the amount of formulation dropped on the microbalance was recorded as a function of time.

Measurements without mucin were also performed using the inclined plane as such, at the same experimental conditions employed in presence of the biological substrate. The amount of formulation dropped down the inclined plane was recorded by means of LabX software (Mettler Toledo, Novate Milanese (MI), I) as a function of time. The amount remained adhered on the inclined plane was calculated as difference between the amount of formulation loaded on the plane and the amount dropped down from the balance. The mucoadhesion properties were determined considering the amount adhered to inclined plane at plateu value time (150 s). The % mucoadhesive normalized parameter was calculated as follow:

$$\text{normalized mucoadhesive parameter \%} = (\%\,\text{adhered}_{\text{mucin}} - \%\,\text{adhered}_{\text{blank}}/\%\,\text{adhered}_{\text{blank}})$$

This parameter allows to put in evidence the mucoadhesive properties independently of the consistency of the sample.

Table 1
Instrumental parameters used for ESI analysis.

Parameters	[ESI(−)]	[ESI(+)]
Capillary voltage	+3500 V	−3500 V
End plate offset	−500 V	−500 V
Nebulizer	40 psi	40 psi
Dry gas	8 L/min	8 L/min
Dry temperature	350 °C	350 °C
Skimmer	−40 V	40 V
Capillary exit	−128.5 V	147.3 V
Oct 1 DC	−12.0 V	12.0 V
Oct 2 DC	−1.70 V	2.12 V
Oct RF	187.1 V	212.0 V
Lens 1	5.0 V	−5.0 V
Lens 2	60.0 V	−60.0 V
Trap drive	53.9 V	63.8 V