

Evaluation of *in vivo* adhesiveness of hyaluronic acid combined with xanthan gum and carbomer [Karos® throat lozenges] to the oral cavity mucosa in human healthy subjects: a pilot study

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Research note

Keywords: Adhesiveness, carbomer, hyaluronic acid, oral cavity mucosa, xanthan gum.

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Abstract

Objective: The use of a hyaluronic acid-based product has the potential to improve the hydration status of the mucous membranes, especially in the oral cavity. This study has evaluated the in vivo adhesiveness of a product based on hyaluronic acid combined with xanthan gum and carbomer to the oral cavity mucosa in human healthy subjects. The product was administered to 10 healthy volunteers during two tests: in the first participants were asked to abstain from drinking and eating for the duration of the test; in the second participants were free to follow their daily routine. In both tests, samples were taken by brushing the oral cavity immediately after administration of the product, and then after each hour, up to 4 hours. A qualitative and quantitative analysis of the drug was carried out by observation of the samples stained with Alcian Blue at pH 2.5.

Results: In both tests the analysis demonstrated the presence of the product in all collected samples, up to 4 hours after administration of the product, confirming that the combination of hyaluronic acid with xanthan gum and carbomer can provide a long-lasting, durable mucoadhesive hydrogel.

Keywords: Adhesiveness, carbomer, hyaluronic acid, oral cavity mucosa, xanthan gum.

Introduction

The development of mucoadhesive hydrogels represents a rapidly developing sector. Hydrogels are preparations with a high water content (80–90%), made from macromolecular organic substances. Hydrophilic polymer chains, forming a three-dimensional network, can store high volumes of water. High water content and soft consistency make hydrogels very similar to natural living tissue. These properties mean that hydrogels are highly biocompatible and their mucoadhesiveness allows adhesion to the oral and pharyngeal mucosa. Mucoadhesion, i.e., the specific phenomenon of creating bonds during intimate contact between biological surfaces lined by a mucous layer and a mucoadhesive material, is crucial for the formulation of compounds useful for the treatment of oral diseases.

Hyaluronic Acid, Xanthan Gum and Carbomer are anionic polymers with hygroscopic, gelling and mucoadhesive properties. When these three active substances come in contact with water or an aqueous solution, they absorb the liquid and form a hydrogel. The action of these three molecules is of physical nature (formation of a viscous mucoadhesive gel), and it is due to their strong mucoadhesiveness.

Therefore, we hypothesized that the combination of hyaluronic acid with xanthan gum and carbomer in the same product would form a durable hydrogel film, able to diffuse through mucous membranes, thus allowing hyaluronic acid to exert its beneficial long-term effects on the oral mucosa.

Main Text

The mucosa of the oral cavity is composed of a stratified squamous epithelium and a lamina propria, separated by a basal membrane. The epithelium consists of a proliferative basal layer, a partially

differentiated intermediate layer, and a terminally differentiated superficial layer. Epithelial differentiation may also vary according to the region of the mouth. The epithelium can be keratinized, para-keratinized or non-keratinized (1). The surfaces of the mucous membranes are kept hydrated, lubricated and protected by the mucus layer. In addition to capturing microorganisms and particles, the mucus acts as a physical and chemical barrier against external agents and acid secretions from the stomach.

The homeostasis of the oral cavity is preserved by the integrity of mucous membranes lined with mucus, by the constant presence of saliva, by the action of enzymes, and by the production of mucosal immunoglobulins (SIgA). It can often be altered by the action of both internal and external agents, leading to the onset of oral diseases such as mucositis, pharyngitis, and xerostomia (i.e., dry mouth).

Hyaluronic acid (sodium hyaluronate) is a high molecular weight polysaccharide that is widely distributed in body tissues and fluids. It is made of a repeated sequence of D-glucuronic acid and N-acetyl-D-glucosamine, thus belonging to the group of glycosaminoglycans. The molecule is flexible, extremely polar and has a high solubility in water, which is important for the hydration of mucous membranes, and thus for its protective and supportive function. Thanks to its molecular characteristics, this molecule looks like an ideal mucoadhesive polymer (2–5).

Hyaluronic acid also plays a major role in tissue repair and healing, promoting fibrin formation and attracting inflammation mediators to the damaged area. Another important feature of hyaluronate is its viscoelastic property, due to this property it can affect cellular functions leading to changes in the surrounding cells and the extracellular environment. Viscoelastic properties can also slow down the penetration of viruses and bacteria, which is of particular interest in oral diseases (6).

Carbomer is a synthetic polymer that is widely used in controlled drug delivery systems. (7). The molecule contains a high percentage of carboxylic acid groups, allowing it to swell in water and produce a certain viscosity; this characteristic is the basis of its mucoadhesive capacity. The oral hydrogels prepared with carbomer are suitable for dispersing and delivery of minitables or pellets and then they can be used also in paediatric patients (8).

Xanthan gum is a natural polysaccharide whose use is common in the food industry as a stabilizer and thickener. Recent clinical studies (9) on the preparation of a mucoadhesive gel based on dimenhydrinate (DMH) have shown that the addition of xanthan gum to this compound provides better physicochemical and mechanical properties compared to a previous association with hydroxyethyl cellulose.

The residence time of formulations for the treatment of disease of the oral cavity is largely influenced by the combined effects of salivation, the swallowing reflex, speech, mastication and the passage of food bolus. Consequently, in the last years there has been an increasing interest in specific mucoadhesion, i.e. the adherence of a material to a mucosal surface for a certain period of time, a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains (10).

Advances in hydrogel, mucoadhesive polymers made from macromolecular organic substances with a high water content (80–90%), have a rapid rate. Hydrogels are highly biocompatible and their mucoadhesiveness allows adhesion to the oral and pharyngeal mucosa.

Aim, Materials and Methods

The aim of the study was to evaluate the in vivo adhesiveness of a product based on hyaluronic acid combined with xanthan gum and carbomer to the oral cavity mucosa in human healthy subjects.

Ten healthy volunteers (6 females, mean age 28.5 y.), referred to the Ear, Nose and Throat (ENT) Unit of Sant'Andrea University Hospital, Rome, Italy, were enrolled from December 2017 to January 2018. Written informed consent was obtained from all study participants, and all procedures were conducted in accordance with the Helsinki declaration.

The inclusion criteria in the study included enrollment of individuals without distinction of age or sex, able to comply with planned procedures (methods and timings of tests), and with no diseases of the oral cavity. Individuals with previous and/or current oral diseases, subjects with a history of known hypersensitivity to any components of the study product, and those under treatment with other products for the oral cavity were excluded from the study.

The study protocol included two tests, carried out one week apart.

For each test, participants were asked to intake the study product (Karos® tablet, sodium hyaluronate) and then underwent sample collection by brushing the oral mucosa. The samples were taken immediately after the administration of the product (T0), and then at 1 hour (T1), 2 hours (T2), 3 hours (T3), and 4 hours (T4) after intake. Samples of the oral mucosa were collected, at the different timepoints, from different areas within the mouth in order not to affect subsequent samples.

In the first test (Test 1), participants were asked to abstain from drinking and eating for the whole duration of the test; in the second test (Test 2), participants were instead allowed to follow their daily routine, so they were free to eat and drink at will.

After each collection, the sample was transferred to a slide and then fixed. Slides were sent to the respective laboratory for cytological evaluation.

The qualitative and quantitative analysis of the compound was carried out by observation of samples under microscope after cytochemical staining with Alcian Blue at pH 2.5.

Densitometric analysis of Alcian blue staining

Oral brush samples were stained with Alcian blue at pH 2.5 (Bio-Optica, Milan, Italy), according to the manufacturer's instructions. For the quantification of the signal, digital photomicrographs of all samples were obtained with an Axiovert inverted optical microscope (Zeiss, Oberhocken, Germany) equipped with AxioCam CCD camera (Zeiss) and Axiovision image analysis software (Zeiss), using a random sampling

method. All slides were digitally scanned and densitometrically analyzed with the dedicated Axiovision software (Zeiss).

The sample area with positive staining for Alcian blue was expressed in $\mu\text{m}^2 \pm$ Standard Deviation (SD); the color intensity was expressed in arbitrary units (AU, mean \pm standard error [SE]), corresponding to the brightness values of the dye on a gray scale from 0 to 256 (max value).

Statistical Analysis and Results

Analysis of statistical significance for quantitative variables was performed by Student's t-test for paired data; the statistical significance threshold was defined as $p < 0.05$; the results of the study are shown in Table 1, and in Figs. 1–2.

Table 1
Densitometric analysis of Alcian blue.

	T0	T1	T2	T3	T4
Test 1 (fasting)	44.2 \pm 5.6	33.2 \pm 4.1	36.1 \pm 4.6	34.8 \pm 4.3	35.3 \pm 4.5
Test 2 (normal routine)	45.4 \pm 4.3	38.1 \pm 2.6	36.6 \pm 1.6	31.9 \pm 2.8	31.4 \pm 3.8
Values are expressed as Arbitrary Units staining intensity (mean \pm Standard Error; reference intensity of untreated sample: 34.2 AU)					

The results of this preliminary study demonstrate that the combination of hyaluronic acid, xanthan gum and carbomer has adhesive properties on the oral mucosa, satisfying the adhesion criteria of the classic mucoadhesive hydrogels. The effective adhesive duration of this product, based on hyaluronic acid, xanthan gum and carbomer, is at least 4 hours. Based on a quantitative analysis of time-intensity data, both in the fasted state and in the normal fed state, product adhesiveness does not appear to be significantly affected by the intake of solid foods and/or drinks.

The combination of hyaluronic acid, xanthan gum and carbomer is more effective, in terms of duration, than the individual mucoadhesive polymers; accordingly, this product may have positive effects on the mucosa of the oral cavity as a result of the long-lasting effect of hyaluronic acid, including better hydration, improved repair capacity of the mucosa, and a protective effect against internal or external insults.

Limitations

The limitations of the study were:

- A restricted cohort of 10 subjects;
- Participation restricted to healthy subjects, with no previous and/or current oral diseases;
- Participation restricted to subjects not under treatment with other products for the oral cavity.

Abbreviations

AU:Arbitrary Units; Dimenhydrinate (DMH); ENT:Ear, Nose and Throat; SD:Standard Deviation; SE:Standard Error; SIgA:Secretory Immunoglobulin A

Declarations

- Ethics approval and consent to participate: The Ethical Committee on Human Research at the Sant 'Andrea University Hospital deemed the need for ethical approval unnecessary; the present research, in fact, involved a medical device, which was already authorized and in commerce in European market. Furthermore, the research was not intended to test its efficacy, but a pharmacological propriety. Written informed consent was obtained from all study participants, and all procedures were conducted in accordance with the Helsinki Declaration
- Consent for publication: Not applicable
- Availability of data and materials: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study
- Competing interests: GB, RT, GP, AM and SR declare that they have no competing interests
- Funding: Not applicable
- Authors' contributions: GB= Concept, Design and Supervision of the study; RT, GP, AM= Data collection and Processing; SR= Data Analysis and Interpretation, Critical Reviews and Supervision; RT= Literature Search, Design and Writing;*all Authors have read and approved* the final version submitted
- Acknowledgments: Not applicable
- Authors' information (optional): Not applicable

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Figures

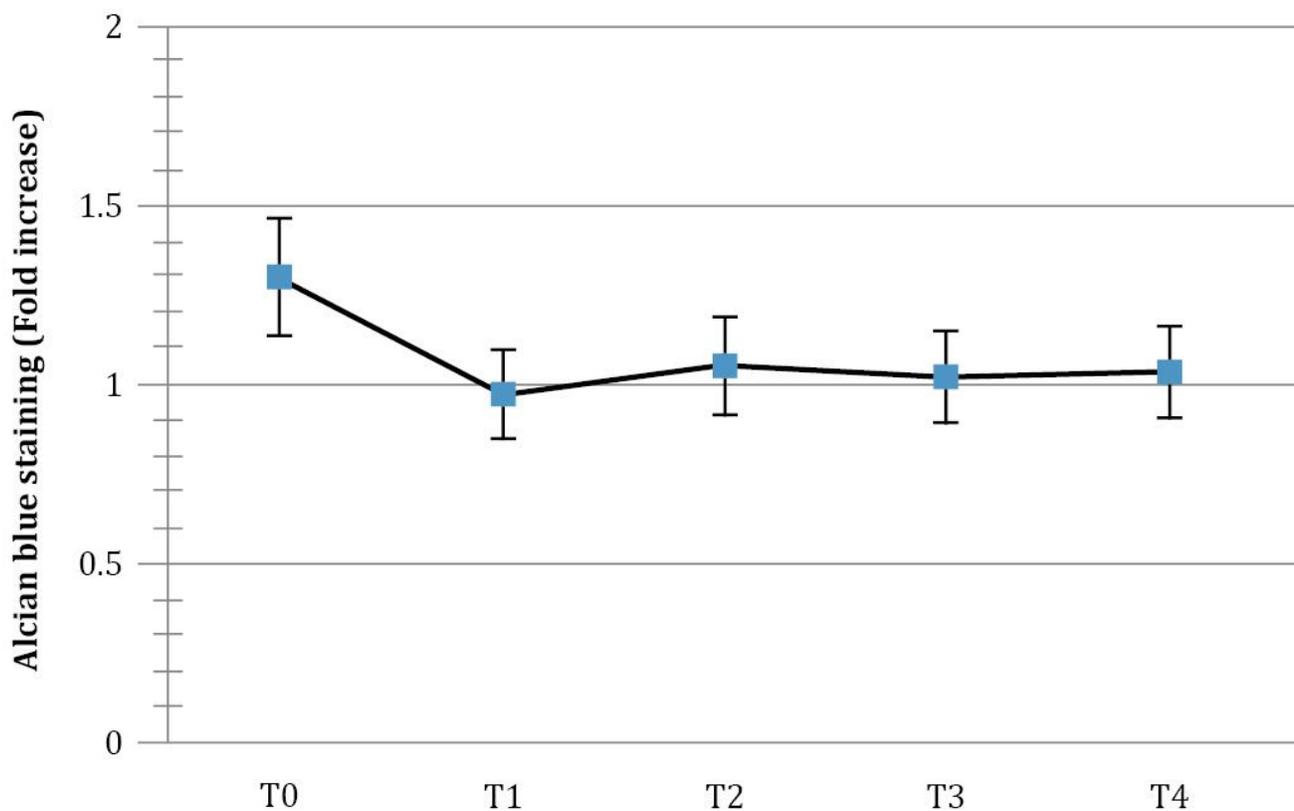


Figure 1

Concentration values of the compound plotted against the duration of adhesion for Test 1 Description: Alcian blue concentrations, expressed as normalized mean intensity values, are plotted on the y-axis; sampling time points from T0 to T4 are plotted on the x-axis.

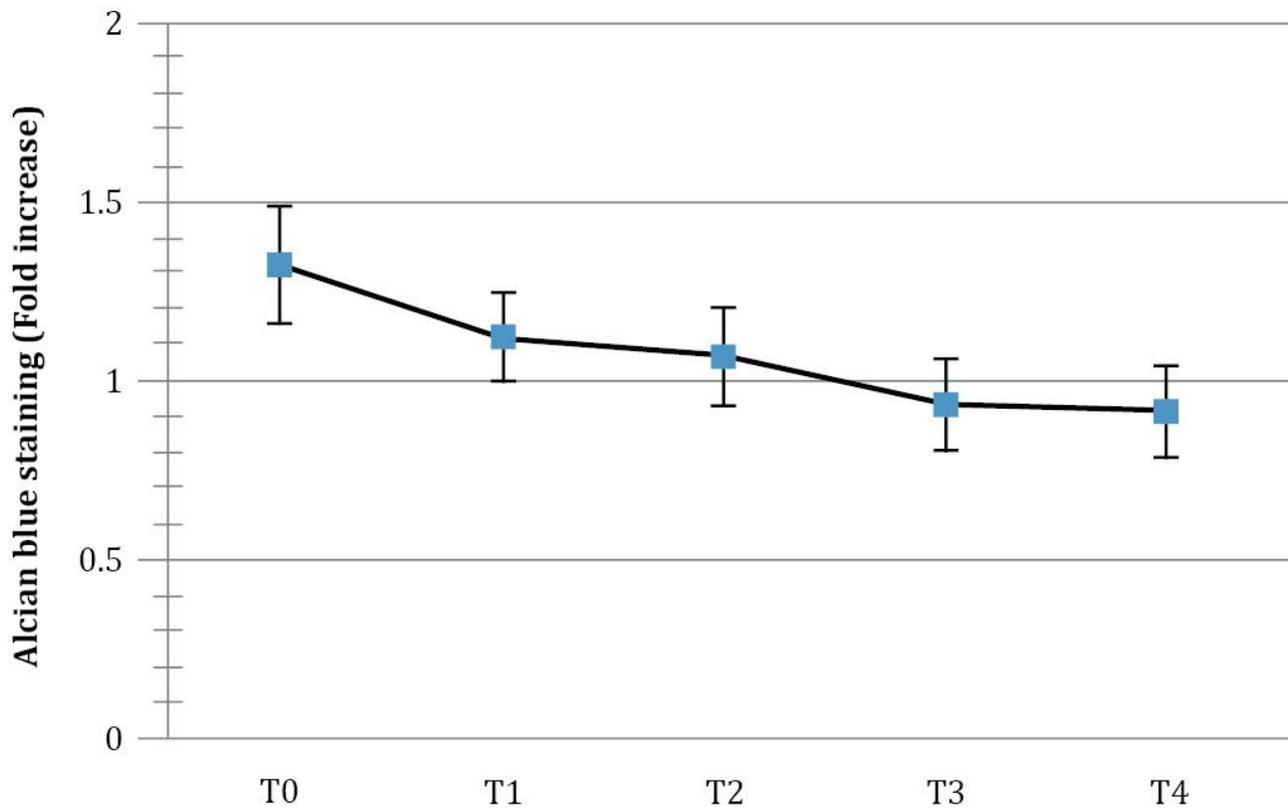


Figure 2

Concentration values of the compound plotted against the duration of adhesion for Test 2 Description: Alcian blue concentrations, expressed as normalized mean intensity values, are plotted on the y-axis; sampling time points from T0 to T4 are plotted on the x-axis.