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Technological development and evaluation on sialagogue activity of a spray-like liquid formulation of pilocarpine

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Xerostomia is a common condition in patients undergoing oncological treatment. As a result of adverse effects of drugs or as an answer to radiotherapy radiation, the salivary glands of mouth stop or reduce the production and secretion of saliva. This leads to severe consequences such as caries, infections, difficulty in swallowing, and sensory loss. Thus, this work aimed to develop a new product to be used in patients suffering from xerostomia and improving salivation added to easy application to promote high acceptance rate. In this way a spray formulation of pilocarpine was developed and evaluated for its in vivo activity (in rats) on salivary stimulation. Pre-formulation, development of spray and quality control studies were performed. The formulation developed was evaluated regarding the ability to improve salivation in adult Wistar rats. There was a significant increase (p<0.05) in salivation produced by spray formulation when compared with oral solution in the same concentrations. The spray formulation is an important tool developed for the treatment and support of patients suffering from xerostomia and optimization of these results should be performed.

Key words: Pharmacology, technology, mouth dryness, pilocarpine, xerostomia, oncology.

INTRODUCTION

Xerostomia is a subjective sensation of dry mouth resulting from a decrease or cessation of salivary glands function with changes in quantity or quality of saliva. Salivary hypofunction is characterized by a quantitative decrease in salivary flow, when it drops to less than 50%, or by a change of saliva composition with loss of mucin, and consequently, reducing lubrication (Coimbra, 2009). It is one of a set of signs and symptoms resulting from certain diseases or various stimuli, represented mainly by irradiation of head and neck, or other cancer treatments, as well as Sjögren’s syndrome, Graft-Versus-Host disease and adverse effects to certain drug therapies. Some systemic diseases can also cause salivary dysfunction, including diabetes, human immunodeficiency virus infection (HIV), Parkinson’s disease, Alzheimer’s disease and cystic fibrosis (Fávaro et al., 2006; Imanguli...
Xerostomia has implications not only physically but also psychologically and socially, with some discomfort for patients due to the feeling of dry mouth and also with the higher number of infections of the oral mucosa and dental caries (Feio and Sapeta, 2005). Xerostomia results in difficulty in swallowing and articulation of words, and a general decline in the ability to eat, talk and sleep (Tolentino et al., 2011). The treatment of these symptoms includes maintenance of hydration, avoidance of tobacco and alcohol, maintenance of good oral hygiene (brushing, chlorhexidine and fluoride to prevent cavities and plaque), and stimulation of the reflex arc, for example, chewing gum with flavors and sugar acids, suited to induce salivation, and also the use of artificial saliva two to three times daily (Coimbra, 2009; Kaluzny et al., 2014).

Finally, the possibility of using cholinergic agents as pilocarpine and cevimeline for stimulating salivation has always been attractive. These agents are cholinergic agonists, acting on M₃ receptors, predominantly expressed in smooth muscle and glandular tissues (Ishii and Kurachi, 2006). This leads to glandular secretion by difference of charges between the spaces in and out of salivary lumen, a process mediated by changes in [Ca⁺²], by the IP₃-mediated Ca⁺² signalling pathway (Nakamura et al., 2004). When there is still some residual salivary function, saliva stimulants produce greater relief than saliva substitutes or other palliative procedures (Kaluzny et al., 2014).

In the past, pilocarpine has been investigated as a mean of systemic management of xerostomia secondary to irradiation of the head and neck. Currently, systemic pilocarpine is indicated for the management of xerostomia secondary to irradiation damage, chronic Graft-Versus-Host disease and glandular autoimmune attack given by Sjögren’s syndrome (Fávaro et al., 2006; Agha-Hosseini et al., 2007). Furthermore is the sole sialagogue agent approved by FDA for radiotherapy treatment (Kaluzny et al., 2014). Cevimeline, another cholinergic agent, was assessed on its sialagogue activity and compared to pilocarpine. Both drugs showed effect on submandibular and sublingual glands, while cevimeline had stronger side effects in central nervous system (CNS) (Omori et al., 2003). Cevimeline activates common salivary mechanism with pilocarpine, but has a slower onset of activation, longer duration of salivation and an increased pressor response at higher doses. However, the cevimeline has an anti-dipsogenic effect due to the inhibitory neuronal effect on the thirst-related central nuclei (Ono et al., 2012).

Pilocarpine treatment in patients with Sjögren’s syndrome usually starts with 5 mg for a few days, then 5 mg twice daily, for a week, and then, if the patient does not respond, the dose is increased to 15 or 20 mg a day. In some cases the dose can be increased to 30 mg a day (Tsifetaki et al., 2003). Some studies with pilocarpine show that the clinical side effects pointed to a small proportion and is usually characterized by facial flushing, sweating and increased urinary frequency, lacrimation and rhinitis (Nakamura et al., 2009; Bernardi et al., 2002; Kaluzny et al., 2014).

An alternative to avoid these effects would be a local than systemic application of the drug. However, the major difficulty in pilocarpine use does not lie primarily in their side effects, but in adoption of a protocol for these patients and the acquisition of drug. The tablet Salagen® is the only formulation with pilocarpine available on market and it is not sold in Brazil. It demands importation and taxes relative to product, making the treatment very expensive (Neto and Sugaya, 2004).

In Brazil, there are no medications based on salivary stimulation, even with pilocarpine, which has effective action in xerostomia and with significant production in the country, including exportation by VegeFlora Ltd. Company, located in Parnaiba, city from Piauí coast located in Brazil. Thus, the development of national products based on this active principle would result in lower costs and higher compliance, improving quality of life for patients suffering from these symptoms.

The aim of this study was to develop a new presentation for pilocarpine based on a spray formulation. Then to realize a pre-clinical trial using rats, aiming to evaluate the spray efficacy regarding to an oral solution of pilocarpine, to compare the spray formulation with a solution representing the current formulation in the market (the oral tablets labelled as Salagen®). This methodology of sialometry aims to evaluate answers (increase or decrease in salivation), as a simple test that can be used in further studies.

MATERIALS AND METHODS

Development of spray formulation

The development of the formulation initially involved the choice of excipients, determined by the capability to increase the duration of drug action and to improve the viscosity, taste and flavor of the formulation. The pilocarpine hydrochloride (active ingredient) was obtained from VegeFlora Company (Parnaiba city, Piauí, Brasil) and its identification was carried out by the method of Fourier transform infrared spectroscopy (FT-IR Spectroscopy) in IR Spectrum 100 brand PerkinElmer KBr cell apparatus, with the range 4000 to 450 cm⁻¹. The others excipients (Honey, methylparaben, propylparaben, glycercin, saccharin, sodium cyclamate, menthol and Hydroxypropyl cellulose - Klucel®) used in formulations were purchased in a manipulation pharmacy, located in Teresina, city of Piauí, Brasil. Alcohol was used in very low quantitative, just to facility the preservatives solubilization. Later, the excipients were mixed at room temperature.

The following parameters were evaluated: organoleptic characters, pH (in equipment brand "Hanna," Model PH21), density (second method described in general methods of the 5th edition of Brazilian Pharmacopoeia, with the help of pycnometer with a capacity of 5 ml), sprinkling volume (50 sprinklings in the bottle valve, driven in a graduated cylinder to measure the corresponding volume) and assay (high resolution chromatography (HPLC), with column LiChroCARTSuperspher 125-4 100 RP-18 end capped, and
Spray:
3 spray (0.3 ml);
Time until sialometry: 10 min;
2, 4 and 14 µmol/kg

Oral:
0.3 ml of pilocarpine solution;
Time until sialometry: 30 min.
2, 4 and 14 µmol/kg

Sialometry:
Initial weight of cotton;
Cotton inserted into the oral cavity;
Last weight, after 7 min;

Standard:
Cleaning of mouth with cotton;
Administration of vehicles for both formulations;
Time until sialometry: 10 min for spray and 30 min for oral solution.

Figure 1. Sialometry study represented in a chronological flowchart of actions.

The active principle was evaluated by infrared as shown in Figure 2, being obtained as a spectrum with intense absorption bands between 2800 and 3200 cm⁻¹, 1600 and 1800 cm⁻¹ and a last one between 1100 and 1035 cm⁻¹. The pilocarpine formulation was developed as shown in Table 1. The formulations evolved until pilot 3, which shows best results regarding to quality control tests and organoleptic characterises and therapeutic necessities by future patients.

With the formulation chosen, tests regarding to quality control were realized to determine its characteristic to assure that it was utilized as a specific and adequate formulation in preclinical test. Then the sialogogue activity test was performed and plotted as shown in Figure 3. In this picture four groups were plotted for three different concentrations of pilocarpine (2, 4 and 14 µmol/kg). These groups include the placebo for both formulations (oral solution and spray), and the two formulations with their respective concentrations. The figure shows the...
gain and stop of gain in salivation for both formulations. Added with physiological behaviour observations, these results allow to compare which has more efficacy and safety. The results were analysed statistically by ANOVA and t-Student-Newman-Keuls as post hoc test.

**DISCUSSION**

In infrared spectrum, the intense absorption band between 2800 and 3200 cm\(^{-1}\) suggests similarities with the amine attached to aromatic carbon representing the substituted imidazole ring. Another absorption band was observed in 1770 cm\(^{-1}\) which means typical double bond between carbon and oxygen (C = O) in lactone rings, and in 1620 cm\(^{-1}\), C = C bond of the aromatic type, both indicating the presence of the second ring constituent of the pilocarpine hydrochloride molecule (Silveira, 2010). The pilocarpine analysed was in its hydrochloride salt presentation, which was evidenced by the presence of the peak between 1100 and 1035 cm\(^{-1}\), representing the connection between carbon and chloride in aromatic ring (Pavia et al., 1996).

After confirming the presence of active principle, the study continued with development of a spray formulation as shown in Table 1. The formulation should contain ingredients to promote safety and adhesion to treatment. Thus it has the presence of preservatives, sweeteners and vehicle, resulting in an aqueous formulation, colourless and with sweet taste.

Saccharin is 300 to 600 times sweeter than sucrose and frequently used in tablets, oral care products and oral
pharmaceutical formulations, in concentrations from 0.02 to 0.5% w/w (Rowe et al., 2009). However, in approximately 25% of the population saccharin leads the feeling of metallic or bitter taste, even in normal doses, which can be masked by the addition of other sweeteners agents in low concentrations. Therefore, a second sweetener was included, glycerine, which is clear, odourless and approximately 0.6 times sweeter than sucrose. Besides, glycerine is a sticky and hygroscopic agent, giving also a higher viscosity to the formulation (Rowe et al., 2009).

Beside this was introduced honey and sodium cyclamate, two sweeteners. The choice of honey was influenced by its agreeable aroma and flavor, and also medicinal properties have long been known. When applied to the oral mucosa of patients undergoing radiotherapy for example it appears to offer an additional benefit, limiting the severity of mucositis, often presented by these patients. In another study, patients with head and neck cancer were treated with honey, exclusively, with a significant reduction in the discomforting symptoms of mucositis (Bardy et al., 2008).

Therefore, honey was added for its healing properties, stimulating tissue growth, anti-inflammatory and antibacterial properties, reducing the discomfort and the emergence of infections (caries, gingivitis, etc.), especially in irradiated patients (Khanal et al., 2010).

The addition of hydroxypropyl cellulose, a bioadhesive polymer, was used to work like a matrix for controlled release of drugs. Formulations designed for delivery to the mouth have the problem of high swelling, which leads to a low contact time between drug and surface. Thus, its mucoadhesive property drew attention to the benefits of forming a film on the oral mucosa, prolonging the local effects of the drug on the salivary glands (Rowe et al., 2009).

As the aroma directly affects the reflex response to increased salivation, to make it even more pleasant and attractive, was introduced menthol. It is a flavouring agent that gave a pleasant aroma to the formulation, exerting a fresh feeling probably by direct interaction with cold receptors in the body, a fact exploited in most commercial topical presentations, mainly oral (Rowe et al., 2009).

The formulation presented sensory parameters of pleasant smell of menthol and honey, sweetness flavor and cooling sensation, and optimum viscosity to remain longer on the oral mucosa, as compared with liquid formulations, which have low viscosity and tend to be swallowed faster. The pH was 3.45 ± 0.12, below the normal pH of the oral mucosa which was determined by the presence of acidifying agents such as sucrose (pH = 2.0 in 0.35% w/v), hydroxypropylcellulose (pH = 5.0 to 8.5 in 1% w/w), sodium cyclamate (pH=5.5 to 7.5 in 10% w/v) (Rowe et al., 2009). However, it is an oral formulation that should be compatible with physiological pH, avoiding discomfort, irritation or even damage to the mucosa with the drug (Bhanja et al., 2010). The physiological pH of mouth is kept within the range of 6 to 7. However may vary between 5.3 (at low flow rates) and 7.8 (in peak salivation) (Humphrey and Williamson, 2001).

Despite the possibility of use of lemon juice or citric acid 2% on the back of the tongue to stimulate salivation in normal conditions, the xerostomia patients already suffer with the consequences of acidity (Feio and Sapeta, 2005). To avoid this potential problem, is important as the addition of sodium hydroxide until the adequate pH in the

![Figure 3. Sialometry analyse in Wistar rats of pilocarpine spray and pilocarpine in oral solution. aP < 0.05 when compared to the lowest concentration; b P < 0.05 when compared to the intermediate concentration; c P < 0.05 when compared to the lowest and higher concentrations, * P < 0.05 when comparing vehicle to any formulation: (ANOVA and t-Student-Newman-Keuls as post hoc test).](image)
range of 6 to 7.

The bulk density obtained was 1.1022 g/ml, above the density of water. The volume for sprinkling, important to determine the dosage of the new medicine, resulted in 0.1 ml/sprinkling. Three spray formulations were produced, named F1, F2 and F3. The theoretical concentrations of the three formulations were equivalent to the doses of 2, 4 and 14 µmol/kg, according to previous studies in xerostomia. Adjusted to the average weight of the adult rats (280 g), and the dose administered to each one determined as three sprinklings (0.3 ml), resulted in formulations with 0.45, 0.91 and 3.2 mg/ml of pilocarpine, respectively.

To confirm this theoretical concentration, an assay study made in high performance liquid chromatography (HPLC), showed that F2 had approximately two times more active than F1 and the F3 had 3.5 times than F2. Thus no interaction occurred between the active and the excipients, making the formulation appropriate for the drug in use and able to be performed in sialometry study. With the spray formulation already developed, the test of sialogogue activity was performed with results as shown in Figure 3. First, the statistical analysis of placebos from oral solution and spray showed a significant increase (p < 0.05) in salivation produced by the second, justified by the advantages given by its excipients, which act as adjuvants in salivary stimulation acting in salivary reflex. These excipients are represented mainly by the honey and menthol. The attractive aroma and the sweet supply (honey) present in the formulation, induce the gustatory memory, with the emergence of primitive reflex responses such as licking the lips and saliva (Guyton and Hall, 2006) which was observed in healthy rats, without damage to the salivary glands.

An oral solution of pilocarpine and water, a systemic presentation, were used to compare with the spray, the local presentation. The advantage of the spray can be observed in the significant increase (p <0.05) obtained with the concentration of 4 µmol/kg compared to the oral solution. When comparing the group of 14 µmol/kg spray with the lower doses (placebo group, spray 2 and 4 µmol/kg) the salivation increased significantly. However, the variation between responses in doses of 4 and 14 µmol/kg was very small. Higher doses should be avoided when there is no comparable improvement and more side effects are likely to emerge (Santana, 2009).

In the oral solution, a significant increase of salivation was observed with greater dose compared to the other groups of oral solution and placebo. However, this variation was not linear. The oral group of 14 µmol/kg had greater than the acceptable variation within the population sample, with a standard deviation of ±45. The error in this group suggests the exaggerated increase in stimulating effect (excitatory) that the systemic concentration produces in central nervous system (CNS). Among the typical side effects, the animals developed diarrhoea, increased urination, cardiac abnormalities, and muscle contractions in some rats, which have been cited in the literature like toxicity effects of cholinergic agonists (Santana, 2009).

In this way, the spray formulation showed better results in lower doses. Despite its lower effects in 14 µmol/kg dosages, it did not present side effects like the oral solution, which systemically led to intolerable side effects. Thus, when compared with the oral solution and the spray in 4 µmol/kg, a significant increase was observed demonstrating the efficiency and quickly action of oral topical formulation.

**Conclusion**

The spray formulation of pilocarpine hydrochloride, with all the excipients chosen, showed promising results to induce salivation in preclinical studies, with better results in lower doses and even with high doses showed more safety than oral solution with the same active drug. A patent application has been made with the INPI. Clinical trials will be conducted to ensure this application.

**REFERENCES**


