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Original Research Article

The effects of cortocosteroids in aerosol inhalation on the protective layer of oral cavity and larynx

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Abstract

The aim of this study is to verify the effects of two corticosteroids usefully employed as aerosol in many respiratory diseases, on salivary protein that have a fundamental rule, on defense of the oral cavity and larynx. Two whole saliva samples were provided by volunteers and they were added in increasing amounts of two types of two corticoseroids drugs. The first corticosteroid, employed in a normal dosage, precipitate a maximum of 24% of the proteins and the second corticosteroid with a maximum of 22%. The results of the precipitation kinetics, for both corticosteroids, depending on the concentration and the pH of the medium, are statistically analyzed by Fisher Exact. These results indicate that the values are not statically correlated in increasing concentration of both corticosteroids, (p≥0.05), but exist as a trend in this phenomenon (rho = 0.9-0.91, and rho = 0.77, -0.88 respectively). In the same experiments the results indicate a statistical difference (p ≤0.05) in the precipitation of the salivary proteins among the two corticosteroid, and for both in function of initial salivary pH. The most likely explanation concerning the different affinity for the corticosteroids in binding sites of the chain, also depending on the pH. These results indicated an undescribed danger for oral cavity defense in the use of corticosteroids beyond skis collater effects.

Key Word: Aerosol Saliva, Corticosteroid, Protein

INTRODUCTION

For its anti-inflammatory, cortisone medications are used successfully in the treatment of respiratory diseases such as asthma, pulmonary fibrosis and COPD. The pharmacology of corticosteroids is very complex, but can be summed up with the explication of these biochemical activities:

a- interaction and complex formation Corticosteride - protein Glycosylated (GR) - (CCP)

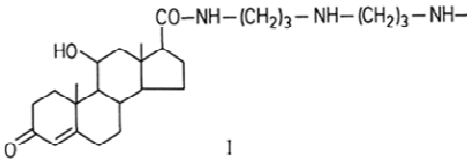
b-transport inactivated form of (CCP) to the target cells

c-penetration (CCP) in the cells and binding to the receptor cytoplasmic GR

d- Anti-inflammatory cortisonic activity

The complex (CCP. Penetrates into the nucleus and interacts with the DNA activating or inhibiting gene transcription, responsible of the major pharmacological effects of corticosteroids. The glucocorticoid-GR complex, in addition, is able to block the way of NFkB, (nuclear factor kappa-light-enhance of activated B cells, the key of many mechanisms in inflammatory and immunological diseases). At this level are activated probably most of the anti-inflammatory actions

Also the results of the studies that have elucidated the mechanisms that lead in human blood to the formation of a "protein -Corticosteroide" complex (see picture 1), that allows the transport of corticosteroids,have a an important rule for the motivation of this work.



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The training involving approximately eighty percent of the concentration of the corticosteroid, is via the link

Table 1. Metabolic activity of oral corticosteroids

COMPOUNDS	GLICOACTIVITY' CORTISOL = 1	*MINERL-ACTIVITY'*	DOSE mg/day	DOSE eq /20 mg Cortisol
Cortisone acetate	0.8	++	25-200	25
Cortisol	1	++	20-200	20
Prednisone	3.5	+	5-50	5
Prednisolone	4	+	5-05	5
Metilprednisolone	5	0	4-40	4
Desametasone	30	0	0.5-10	0.75
Betametasone	33	0	0.6-6	.6
Parametasone	10	0	2-20	2
Fluprerdnisolone	15	+-	2-15	1.5
Fludrocortisone (9αfluoro idrocortisone)	20	+++++	0.1-0.3	1
Trimeinolone	5	0	4-40	4

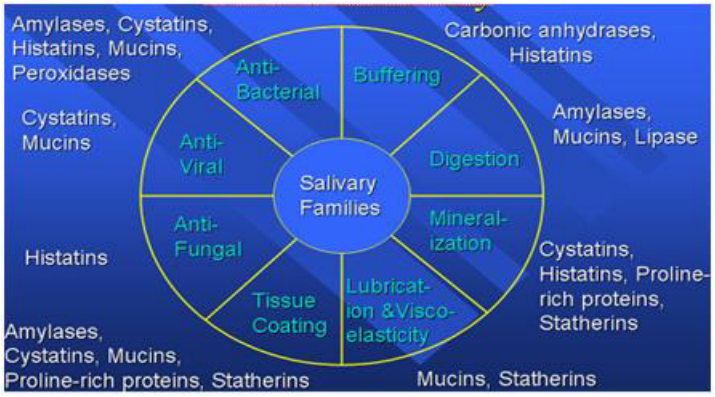

Figure 1. The principal functions of salivary proteins

between the same (position C19 and C 23), and a protein that is a non-inhibitory member of the serine proteinase inhibitor (serpin) super-family,and have high-affinity transport protein for glucocorticoids in blood. Plasma. CBG is a glycoprotein with 30% of its mass represented by N-linked oligosaccharide chains (Chuang C K.2014). Recent crystal structure analyses of intact rat CBG and cleaved human CBG have revealed the precise topography of the steroid-binding site, and shown that cortisol-bound CBG displays a typical stressed (S) serpin conformation with the Reactive Center Loop (RCL) fully exposed from the central beta-sheet (1) (Gardill et al., 2014; Lyn, 2012). Corticosteroids can also pair with other proteins and their affinities can vary greatly (Westphal, 1978). In function of many parameters. These bonding mechanisms have been studied in order to understand both the anti-inflammatory capacity of cortisone is some of the possible side effects of these drugs. This key factor comes from the fact that the individual corticosteroids can bind to more blood proteins and have consequently different metabolic pathways of activity and / or detoxification, as can be seen also in table 1, the 'pharmacological activity.

These variations actually depend not only on the type of chemical structure of cortisone compounds, but as it has been shown recently also, and perhaps more than that of proteins. In particular may occur naturally or not, modifications / alterations in the glycosylation process of the serum proteins, which involves a variation in the percentage of the carbohydrate part (Avvakumov, 2002; Ali, S; Basset Jr, 1998). These changes modify the protein folding in a definitive way with consequences both in terms of possible catalytic activities of proteins. Both in the processes of interaction of the same, with different substrates as it is clear that the process and interaction / binding between proteins and corticosteroids easily occurs in the blood. With the formation of a stable complex, that reasonably can also happen in the saliva. In this medium, also is not present globulin, which in the blood has the highest affinity with corticosteroids, and therefore they can bind with various proteins present. This fact can change their functionality and their role is critical to the defenses of the oral cavity and larynx, as you can see from Figure 1

Beyond the types of nebulizers used in aerosol therapy, it must be emphasized that it is not associated

Figure 2. Saliva samples

only in the winter season and with colds, but also the spring and allergic phenomena, which occur more and more numerous in this season. Therefore the constant use of corticosteroids may lead to increased side effects because of the latter, they should be used with caution. Cortisonic drugs often inhale can lead to hoarseness, with atrophy of the vocal cords and xerostomia. At these aspects, the aim of this study is to verify if during the therapy aerosol- salivary proteins are precipitated by two different corticosteroid drugs

EXPERIMENTAL APPARATUS – METHODS

Two whole saliva samples, for boyh 10 ml, were provided by male volunteers aged respectively 30 and 60 years, who are not smoking, not denounced cardiovascular diseases the amounts of the samples of saliva are those produced in an average time it takes for one cycle of the aerosol therapy.

In saliva samples before and after the addition of corticosteroids, were determined the concentrations of total protein by the biuret test at, and the pH

Two samples of corticosteroids, drugs and their commercial properties are as follow:

1-beclomethasone dipropionate: 0.8 mg / 2 ml suspension to be nebulized aerosol
Single-dose vials of 2 ml aerosol
Composition
100 ml of sterile suspension containing:
Active ingredient: beclomethasone dipropionate 0.040 g.
Excipients: Sodium chloride; Polysorbate 20; Sorbitan monolaurate; Water for injections
2- Budesonide 0.5 mg / ml suspension nebulizer 1 single-dose container contains:
Active ingredient: 1 mg busedonide
The results were statistically analyzed by the Fisher Exact Test

RESULTS

The addition to the two whole saliva samples of beclomethasone and busedonide, at low concentrations, ≤ 0.4 mg / ml, involves the formation of an opalescent-colloidal solution that after centrifugation door wing formation of two phases, a liquid and a solid as can see in Figure 2.

. In highest concentrations in a short time, about two minutes, the separation of the two phases, see picture, even for the analysis is still a work centrifugazione. In

Table 2. Comparison of two corticosteroid for the sample of whole saliva number 1

COMPOUND mg/ml	Salivary Proteins mg/ml	pH initial	pH final	Ratio beclometasone/salivary protein	Salivary proteins in surnatant mg/ml	Percentile (%) precipitation
Beclometasone						
0.16	25	7.4	7.1	0.065	17	32
0.8	25	7.4	7.1	0.033	18	26
0.6	25	7.4	7.3	0.022	21	16
0.4	25	7.4	7.4	0.016	22	12
0.2	25	7.4	7.4	0.008	24	4
Budesonide						
0.16	25	7.4	7.2	0.065	17	29
0.8	25	7.4	7.2	0.033	19	22
0.6	25	7.4	7.3	0.022	20	16
0.4	25	7.4	7.4	0.016	22	9
0.2	25	7.4	7.4	0.08	23	6

1-Beclometasone Drug: relationsheep concentration/ protein precipitation
Fisher Exact Test p≥ 0.05; rho = 0.9
2-BUDESONIDE DRUG: relationsheep concentration/ protein precipitation
Fisher Exact Test p≥ 0.05; rho = 0.77
3- Relationsheep Beclometasone /Budesonide p≤ 0.05

Table 3. Comparison of two corticosteroid for the sample of whole saliva number 2

COMPOUND mg/ml	Salivary Proteins mg/ml	pH initial	pH final	Ratio% beclometasone/salivary proteins	Salivary protein inSurnatant mg/,ml l	Proteins (%) precipitation
Beclometasone						
0.16	32	6.2	6.5	0.0	24	25
0.8	32	6.2	6.3	0.0	26	19
0.6	32	6.2	6.3	0.0	28	12
0.4	32	6.2	6.2	0.0	30	6
0.2	32	6.2	6.2	0.0	31	3
Budesonide						
0.16	32	6.2	6.5	0.005	23	24
0.08	32	6.2	6.5	0.0025	27	17
0.06	32	6.2	6.3	0.002	29	9.5
0.04	32	6.2	6.2	0.0013	30.5	5
0.02	32	6.2	6.2	0.0007	31	3

1-beclometasone drug: relationsheep concentration/ protein precipitation
Fisher Exact Test p≥ 0.05; rho = 0.91
2-Budesonide drug: Relationship concentration/ protein precipitation
Fisher Exact Test p≥ 0.05; rho = 0.88
3- relationsheep beclometasone/budesonide : p≤ 0.05

surnants, has been determined the concentration of total proteins and pH. the results are given in tables 2 and 3, with the statistical analysis of the data obtained.

Theese results show that the increased concentration of the both corticosteroid, results in a tendency to the increase in the precipitation of salivary proteins,regardless of the initial values of pH and protein concentration The correlazionetra increased concentrations of corticosteroihdi however, is not statistically significant, p≥0.05, while the values of rho clearly show that in a fairly wide range, the precipitation of proteins is influenced by ' increase in the concentration of the drugs.The difference in precipitation of the proteins from the two drugs, having an active principle other, is statistically significant, p≤ 0.05 regardless of both saliva sample

DISCUSSION

The results of this study clearly indicate that regardless of the type of corticosteroid used in the application of the

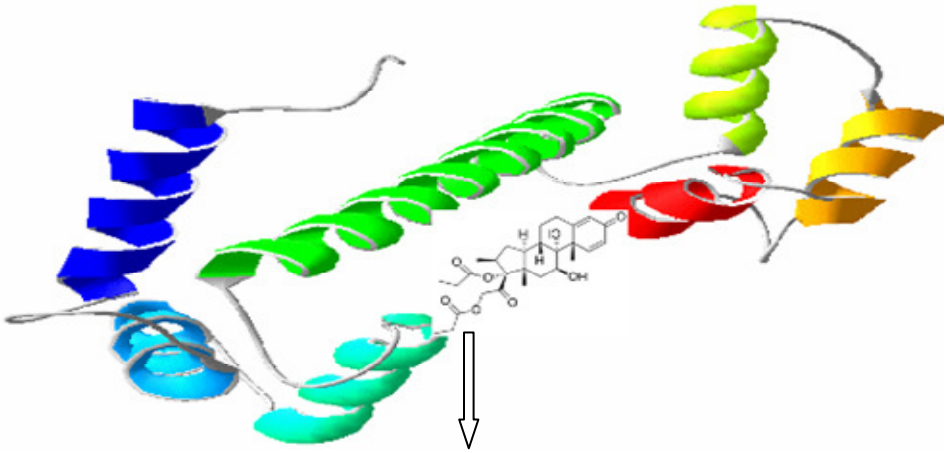

Figure 3a. First phase: Interaction of beclometasone with proteins

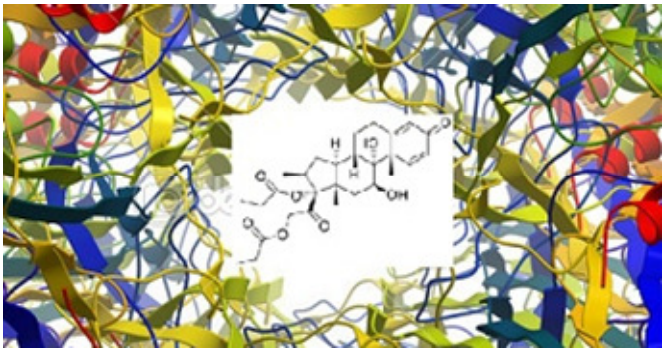

Figure 3b. Second phase: New structure conformation of salivary "protein- beclometasone" coniugates and precipitation

aerosol, there is a precipitation of salivary proteins. It has been widely studied, and clarified, the mechanism of the interaction between blood serum proteins and corticosteroids, a phenomenon that allows the transport of the latter in the blood circle.This mechanism, depends on many factors: the nature of corticosteroids, which establish their greater or lesser affinity with proteins, the structure of the same protein, and more particularly their conformation relatively to the degree of glycosylation, the pH of the blood and the ionic concentration in the solution. In the blood in each case there will be a phenomenon of precipitation of the complex protein - corticosteroide, because we are in the presence of a circulatory flow very dinamic. In the whole saliva instead, the proteins are in a situation in which the phase of their physiological replacement lasts on average half hour drive (Gibson et al., 2014), for which the corticosteroids compounds can easily interact with them by setting the characteristic of the colloidal structures formed by the interactions between the proteins and dispersed in saliva. The physical properties of colloids and in particular their stability in the dispersing phase, in our case the saliva, depend on the electrical double layer that characterizes the protein of the double layer interface , or generated by the electrical potential, called potential Z. The formation of links between a single corticosteroid molecule and a protein colloidal particle deforms the latter structure, change the interface of the double layer, changing the Z potential and it has the precipitation. In Figure 3 (Menicagli and Duca, 2015), has outlined the process described

It is evident that this phenomenon is different depending on the concentration of corticosteroids, nonce from their nature and this explains the results obtained with our trials. It's also clear that the precipitation kinetics may not have a constant trend, and there is a strict proportionality between the precipitated proteins and dose of corticosteroids, since they will contribute to a certain moment of allosteric phenomenon due to the encumbrance of sedimentazione.So this processes explains the results of statistical analysis of the data we obtained that provide for all experiments a non-statistical significance, p≥ 0:05, including increased concentration of corticosteroids and those related to the amount of protein precipitate.An another result of this study also involves' use of beclomethasone leads to a higher

precipitation of proteine. This result is probably linked to the fact that beclomethasone is employed in the form of salt of propionic acid formulation that increases its liposolubilation, favoring the intracellular access. This characteristic makes it most likely increases the affinity towards salivary proteins for the possible formation of ulterior ties lipophilic type. The pH salivary finally, implies that regardless of corticosteroids clerical protein precipitation is greater for the highest values given. This is explainable, because as one moves away from the isoelectric point of the protein, which is zero for values of pH around the 5, or going to the basicity, they assume a steric conformation more relaxed and therefore more willing to molecular interactions.(Menicagli et al., 201; Menicagli et al., 2016))

CONCLUSIONS

The first conclusion that can be drawn from this study is that the use of corticosteroids administered via aerosol certainly involves a precipitation of salivary proteinsi.This phenomenon in standard operating conditions in the application or aerosol, 0.8 mg / ml of compound sprayed , implies a precipitation of salivary proteins from a minimum of 18% to a maximum of 26% depending on the type of corticosteroid and of the initial conditions of the whole saliva.Thesese quantities in each case are not negligible that if on the one hand fully justify the appearance of some effects side of the therapy such as dry mouth, hoarseness. Fungal infections, on the other hand clearly show that the oral cavity for long periods is deprived from a part of its very natural defenses. It will be very important and this will surely the purpose of a subsequent study, measure the amount of precipitated salivary mucin in relation to total proteie. With literature data, that show an estimated 20% of the total salivary mucin proteins, we must assume a similar value of precipitation. In actually the conformation of mucins, has a highest concentration of sialic acid compared to the other proteins present in saliva, to effect a greater glicosilazione. The data of literature concerning the affinity of corticosteroids to the serum proteins indicate a lower percentage of interaction between them, but on occasions the glycosilated proteins are the subject of a basic desinilization. In process this data is not unlikely to assume that the fraction mucinica salivary precipitated by steroids, is much higher than 20% theoretical, and this is another important signal of danger in the use of corticosteroids aerosol.The another important question in the use of corticosteroids aerosol regards the pH of saliva. The results obtained in this study show a lower concentration of the precipitated proteins for the saliva to lower pH and this could lead to think to a fact positive. In reality the oral mucosa in the presence of a saliva at low pH has a mucus-protein protection less, and then the drug acts more strongly increasing the anti-inflammatory corticosterods effects but also the collateral effects cited in the literature, and finally and most important those relating to the impoverishment of the mucosal protective layer. This fact is very important but on occasions the use of aerosols is necessary, even in the presence of autoimmune diseases, where these conditions of salivary pH, are present, together with a lower protective layer mucosal intrinsic to the pathology (Duca et al., 2015)

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