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Research

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Effectiveness of 5-Pyrrolidone-2-Carboxylic Acid and Copper Sulphate (Pirometaxine) in Cold Dry Air Induced Rhinitis with or without Viral Superinfection

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ABSTRACT

Introduction: Exposure to cold dry air can determine rhinitis-like symptoms. 5-Pyrrolidone-2-Carboxylic acid (PCA) and Copper Sulphate (CS) showed antiviral and osmoprotectant effect. Aim of this study is evaluation of effectiveness of 5-Pyrrolidone-2-Carboxylic acid (PCA) and Copper Sulphate pentahydrate (CS) (Pirometaxine) association in the treatment of cold dry air rhinitis with or without viral infection.
Methods: Fifty consecutive adult patients affected by cold dry air rhinitis with or without viral/bacterial infection were enrolled in this prospective observational study and treated with pyroglutamic acid (PCA) plus copper sulphate (CS) (pirometaxine). All patients underwent an objective evaluation with optic fiber endoscopy and symptoms assessment at time of enrolment, after 3 and 7 days of treatment.
Results: All symptoms scores decreased at 3 and 7 days with a mean Total Symptoms Score of 5.98±2.22 and 1.30±1.23, respectively. Twenty-seven patients (54%) reached a Total Symptoms Score of zero after 7 days. Median number of days until healing was 6. Only 18% of cases had turbinate hypertrophy and 10% nasal mucosa hyperaemia after 7 days. Main side effects were nasal itch in 4 cases (8%) and nasal burning in one case (2%).
Conclusions: The association of 5-Pyrrolidone-2-carboxylic acid and copper sulphate (pirometaxine) represent a safe treatment for CDA-induced rhinitis with or without viral infection, with few side effects. Clinical importance of this therapy will be better understood with further studies, comparing results to those observed in a control group.

KEYWORDS: 5-Pyrrolidone-2-carboxylic acid; Copper sulphate; Pirometaxine; Cold dry air induced rhinitis; Viral rhinitis; Common cold.

ABBREVIATIONS: PCA: 5-Pyrrolidone-2-Carboxylic acid; CS: Copper Sulphate; CDA: Cold Dry Air; RANTES: Regulated on Activation, Normal T Cell Expressed and Secreted; SA: Staphylococcus Aureus; SE: Staphylococcus Epidermidis; MRSA: Methicillin Resistant Staphylococcus Aureus; FEV_1: Forced Expiratory Volume in one second; SPSS: Statistical Package for Social Sciences.

INTRODUCTION

Rhinitis is characterized by nasal mucosa inflammation and may be caused by many stimuli, including virus, bacteria, allergens, environment condition.

Viral rhinitis is often designated as “common cold” and represents an heterogeneous group of diseases caused by numerous viruses, including rhinovirus, picornavirus, coronavirus, adenovirus, para-influenza virus, influenza virus, metapneumovirus and respiratory syncytial

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virus.[1] It is defined by typical signs and symptoms, such as nasal congestion, postnasal drainage, rhinorrhea, sneezing, sore throat and cough. Other symptoms may also include headache, malaise and lethargy.[2] Common cold is usually a self-limited disease, confined to upper respiratory tract, but sometimes it predisposes to bacterial superinfection or spread to adjacent organs, resulting in different clinical manifestations.

Despite its usually benign nature, common cold is an important economic burden for society in terms of medical costs and days off work. In US total annual economic impact of common cold is estimated to be USD 40 billion dollars.[1]

Common cold is the result of damage caused by virus and host's inflammatory response but detailed mechanisms are still only partially understood.[2] Viral infection of nasal mucosa results in vasodilation and increased vascular permeability, which cause nasal obstruction and rhinorrhea. Cholinergic stimulation leads to increased mucous gland secretion and sneezing. Different viruses cause different degree of epithelial destruction in nasal mucosa: influenza virus and adenovirus induce extensive damage to respiratory epithelium, while rhinovirus doesn't have cytopathic effects.[3] Probably during rhinovirus infection epithelium damage is determined by host inflammatory response with increase of several inflammatory mediators, such as kinins, leukotrienes, histamine, interleukins 1, 6, and 8, tumour necrosis factor, and RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted).

Another stimulus that can determine rhinitis-like symptoms is exposure to Cold Dry Air (CDA). Many subjects experience rhinorrhea and nasal congestion after exposure to cold windy environments.[4] Nasal burning sensation frequently precedes or accompanies these symptoms. Some conditions predispose subjects to be particular sensitive to CDA, for example patients with non allergic rhinitis react to CDA more vigorously than healthy people.[5]

When exposed to CDA, nasal vascular structure performs air conditioning by dilatation of resistance vessels and increasing blood flow. These effects lead to increased evaporation of water from nasal mucosal surface, resulting in increased tonicity and osmolarity of nasal secretion. Hyperosmolar stimuli can trigger nerves directly activating parasympathetic system that cause nasal congestion and rhinorrhea.[6] While viral rhinitis damage is determined by virus itself or consequent activation of immune system, CDA-rhinitis damage is mediated by hyperomolarity.

Currently a causal treatment for common cold is not available, but only general precautionary measures and symptomatic therapy. Promising data could come from 5-Pyrrolidone-2-carboxylic acid (PCA) and copper sulphate pentahydrate (CS). Some studies showed *in vitro* antiviral effect of PCA and its potential use as hands cleanser. In particular, PCA has antiviral long-lasting activity against Rhinovirus Type 39 and against Influenza A virus (H3N2).[7] Rhinovirus inactivation by PCA appears to involve virion structure changes similar to those that occur during virus uncoating. This conformational change results in loss of infectivity.[8] Another PCA intrinsic function is ability to bind water in the skin and mucosa. This effect is useful to counteract damage-induced hyperosmolarity.

Ion copper alone or in complexes have been used for centuries to disinfect liquids, solids, and human tissue. Today copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, and antibacterial.[9]

A recent study investigated a new combination for hand disinfection evaluating effects of PCA and CS upon different bacterial species that normally colonize hands, including *Staphylococcus aureus, Methicillin Resistant Staphylococcus Aureus (MRSA), Staphylococcus epidermidis, Streptococcus pyogenes, Candida albicans*. PCA and CS had a strong microbicidal activity against different bacterial species and a synergistic effect of the combination CS and PCA against all the tested species was observed.[10]

Aim of this study is evaluation of effectiveness of 5-Pyrrolidone-2-carboxylic acid (PCA) and copper sulphate pentahydrate (CS) (pirometaxine) association in the treatment of cold dry air rhinitis with or without viral infection.

MATERIALS AND METHODS

Between February and April 2015, fifty consecutive adult patients affected by cold dry air rhinitis with or without viral/bacterial infection were enrolled in this prospective observational study and treated with pyroglutamic acid (PCA) plus copper sulphate (CS) (pirometaxine) at our Department. Inclusion criteria were represented by cold dry air-induced nasal symptoms, such as rhinorrhea and nasal obstruction. Exclusion criteria were: clinical signs/symptoms of rhinosinusitis, micotic rhinitis, exacerbation of allergic rhinitis due to exposure to allergens, epistaxis, bronchopneumonia. Institutional Review Board approval was obtained and patients gave their written consent.

Treatment dose was two nasal drops per nostril three times a day for 7 days. Disposable vials were used. Other therapies were not administered to patients during the study.

All patients underwent an objective evaluation with optic fiber endoscopy by an ENT specialist at time of enrolment, after 3 and 7 days of treatment. Presence of turbinate hypertrophy and nasal mucosa hyperaemia were evaluated. Symptoms assessment was based on a score (nasal obstruction, rhinorrhea, headache, myalgia, cold sensation, sore throat, cough, sneezing): each symptom was evaluated as 0(absent), 1(slight), 2(moderate) or 3(severe). Total Symptoms Score was the sum of all symptoms scores.[11] All patients reported at least a moderate nasal congestion and rhinorrhea (score 2) at enrolment. One-point reduction in nasal obstruction and/or rhinorrhea af-

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ter 3 days was considered clinically significant and percentage of one-point reduction in these symptoms was considered the primary end-point. A patient was considered completely healed when Total Symptoms Score reached zero (number of days until healing was the secondary end-point).

Moreover a diary was compiled by patients every day, basing on the same symptoms and reporting differences between morning and evening. Side effects were daily reported by patients in the diary.

Statistical Package for Social Sciences (SPSS), version 17.0 was employed for data analysis. A descriptive analysis of all data was performed and they were reported as means or percentages and standard deviations. The Kolmogorov-Smirnov test demonstrated a non Gaussian distribution of variables, so non parametric tests were used. The Friedman test was used to assess differences in the mean of continuous variables and the chi-square test in the mean of categorical variables. A p value less than 0.05 was considered statistically significant.

RESULTS

Mean age was 41.53±10.75 years (range 19-68 years), 28 patients (56%) were male, 21 patients (42%) were smokers and no one had occupational rhinitis. Allergies were reported in 15 cases (30%).

Concerning subjective evaluation at enrolment time, moderate-severe nasal obstruction and anterior rhinorrhea was reported by all patients. Slight-moderate headache, myalgia and sore throat were present in 76%, 68% and 72% of cases, respectively. Cold sensation, cough and sneezing were less frequent. Mean Total Symptoms Score was 10.56±3.63. At fiber optic endoscopic evaluation, the main findings were turbinate hypertrophy and nasal mucosa hyperaemia (100% of patients) at enrolment time.

All symptoms scores decreased at 3 and 7 days with a mean Total Symptoms Score of 5.98±2.22 and 1.30±1.23, respectively (Table 1). Twenty-seven patients (54%) reached a Total Symptoms Score of zero after 7 days. Median number of days until healing was 6 (range 4-10). One-point reduction after 3 days was reached in 70% of cases for nasal obstruction and in 76% for rhinorrhea. After 7 days all patients had at least one-point reduction in nasal obstruction and rhinorrhea. Regarding fiber optic endoscopic evaluation, 72% of patients had turbinate hypertrophy and 50% nasal mucosa hyperaemia after 3 days. Only 18% of cases had turbinate hypertrophy and 10% nasal mucosa hyperaemia after 7 days. Basing on diary, more nasal obstruction and rhinorrhea were reported in the morning, rather than evening.

Statistically significant differences were observed for endoscopic findings, Total Symptoms Score and all symptoms between time of enrolment and after 3 and 7 days of treatment ($p<0.05$). No statistically significant correlation was seen between Total Symptoms Score / number of days until healing and tobacco smoking/allergies ($p>0.05$).

Main side effects were nasal itch in 4 cases (8%) and nasal burning in one case (2%), that generally appeared after one or two days of treatment. No epistaxis was reported by patients. No one stopped treatment because of side effects, which disappeared at the end of therapy administration.

DISCUSSION

Symptoms of rhinitis, such as nasal obstruction and rhinorrhea, are often experienced on exposure to cold windy environments.[12] Some individuals are more sensitive to CDA, for example, patients with non allergic rhinitis react to CDA more vigorously than healthy individuals.[5] A database of 206 individuals with perennial allergic rhinitis and 150 with seasonal allergic rhinitis indicate that cold air is considered a stimulus for nasal symptoms in by 55% and 28%, respectively.[13]

The prevalence of cold air induced rhinitis is not clear, however in a survey of 912 police officers in France, 5.4% reported this complaint and they had a lower Forced Expiratory Volume in one second (FEV_1).[14] CDA rhinitis is considered a possible risk factor for chronic airflow limitation.

Nasal inhalation of CDA causes drying of the nasal mucosa, resulting in increased tonicity and osmolarity of nasal secretions.[6] Hyperosmolar stimuli can trigger nerves directly leading to reflex stimulation of the parasympathetic system with consequent vasodilatation of nasal vessels.[15-17] These effects could lead to increased speed of airflow, increased evaporation of water from nasal mucosal surface and hence increased osmolarity of nasal secretions.

Because increasing the osmolarity of the medium surrounding isolated mast cells and other cells *in vitro* triggers mediator secretion, response to CDA nasal inhalation could also be caused by the release of mediators secondary to an increase in the osmolarity of the mucosal secretions.[6]

During cold air breathing, there is loss of heat and water from mucosal surface, resulting in mucosal cooling and hyperosmolarity of nasal secretions. The affect of cooling of the mucosa is unknown. Evidence has shown that hyperosmolarity is a known trigger for mast cell and sensory nerve activation in the human nose. Water loss leading to hypertonicity is more likely to be the key stimuli compared to heat loss.

Respiratory mucosa of individuals with CDA sensitivity cannot compensate for the loss of water that occurs on exposure to the stimulus, leading to epithelial damage. Cruz, et al. found a 6-fold increase in nasal lavage epithelial cells in the

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Characteristic	Number of subjects (%)			p value
	Enrolment	After 3 days	After 7 days	
Symptoms				
Nasal obstruction				
0 - Absent	0(0)	4(8)	40(80)	p<0.05
1 - Slight	0(0)	22(44)	7(14)	
2 - Moderate	32(64)	20(40)	3(6)	
3 - Severe	18(36)	4(8)	0(0)	
Rhinorrea				
0 - Absent	0(0)	6(12)	36(72)	p<0.05
1 - Slight	0(0)	25(50)	10(20)	
2 - Moderate	35(70)	17(34)	4(8)	
3 - Severe	15(30)	2(4)	0(0)	
Headache				
0 - Absent	7(14)	20(40)	41(82)	p<0.05
1 - Slight	18(36)	23(46)	9(18)	
2 - Moderate	20(40)	7(14)	0(0)	
3 - Severe	5(10)	0(0)	0(0)	
Myalgia				
0 - Absent	13(26)	24(48)	44(88)	p<0.05
1 - Slight	19(38)	22(44)	6(12)	
2 - Moderate	15(30)	3(6)	0(0)	
3 - Severe	3(6)	1(2)	0(0)	
Cold sensation				
0 - Absent	14(28)	34(68)	48(96)	p<0.05
1 - Slight	23(46)	14(28)	2(4)	
2 - Moderate	13(26)	2(4)	0(0)	
3 - Severe	0(0)	0(0)	0(0)	
Sore throat				
0 - Absent	12(24)	24(48)	45(90)	p<0.05
1 - Slight	20(40)	23(46)	5(10)	
2 - Moderate	16(32)	3(6)	0(0)	
3 - Severe	2(4)	0(0)	0(0)	
Cough				
0 - Absent	17(34)	27(54)	43(86)	p<0.05
1 - Slight	20(40)	21(42)	7(14)	
2 - Moderate	13(26)	2(4)	0(0)	
3 - Severe	0(0)	0(0)	0(0)	
Sneezing				
0 - Absent	21(42)	34(68)	45(90)	p<0.05
1 - Slight	19(38)	12(24)	5(10)	
2 - Moderate	8(16)	4(8)	0(0)	
3 - Severe	2(4)	0(0)	0(0)	
Total Symptoms Score	10.56±3.63	5.98±2.22	1.30±1.23	p<0.05
Endoscopic findings				
Turbinates				
Normotrophic	0(0)	13(26)	41(82)	p<0.05
Hypertrophic	50(100)	37(74)	9(18)	
Nasal mucosa				
Pink	0(0)	25(50)	45(90)	p<0.05
Hyperemic	50(100)	25(50)	5(10)	

Table 1: Symptoms and endoscopic findings.

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CDA-sensitive group after CDA, but not after warm, moist air.[18] The difference between CDA-sensitive and insensitive individuals probably relates to the ability of the mucosa to cope with conditions that demand increased water supply to inhaled air or to the epithelial surface. This finding reflects unequal capability of nasal mucosa to interact with environmental temperatures and humidity in individuals.

Cold air is unlikely to be a causal factor initiating respiratory diseases but a symptom trigger. Cold air-provoked nasal symptoms can be effectively treated by nasal decongestants, but their long-term use is discouraged.[19] Anticholinergic nasal sprays markedly decrease cold-air-provoked rhinorrhea, but do not affect nasal congestion or sneezing.[20] These drugs are well tolerated even in long-term use and are therefore suitable for cold air-provoked rhinorrhea. The cold air-provoked nasal symptoms are poorly controlled by histamine-1 receptor antagonists and topical corticosteroids.[21,22]

Infectious rhinitis is usually caused by an upper respiratory tract infection, usually of viral origin. The most common causes are infections due to rhinovirus, coronavirus, adenovirus, parainfluenza virus, respiratory syncytial virus, or enterovirus. Viral infections are generally self-limited and resolve within 7-10 days. Patients with infectious rhinitis typically present with clear to mucopurulent nasal discharge, accompanied by facial pain, olfactory alterations, post-nasal drainage and cough. Persistent facial pain, purulent drainage and fever suggest a secondary bacterial infection.

5-Pyrrolidone-2-carboxylic acid (PCA) and copper sulphate (CS) are known substances with relevant antimicrobial activity. PCA is a natural constituent of vegetables, fruits, fermented soybean and cereals; it is also obtained by cyclization of glutamic acid. PCA can inhibit Bacillus subtilis, Escherichia coli, Enterobacter, Klebsiella, and Pseudomonas strains.[23] Virucidal activity of PCA was reported by Turner and Hendley: PCA, alone and in combination with salicylic acid, has a strong virucidal activity against rhinovirus on human hands. At the concentration of 4 percent, PCA reduced significantly virus recovery and infection rate with residual activity: 15 minutes, 1 hour and 3 hours after contamination, virus recovery from hand were respectively 17%, 28% and 59% while infection rates were 13%, 13% and 22%.[24] Moreover, ability of PCA to bind water and neutral inert nature of this metabolite allow to PCA the profile of osmoprotectant. Indeed PCA represents a very interesting osmoprotectant: high water-binding capacity and the relative metabolic inert nature of PCA makes it a compatible solute and osmoprotectant. Pyroglutamic acid was found to accumulate in response to salt stress and function as an osmoprotectant along with ectoin and sucrose, known osmoprotectant. The de novo synthesis of pyroglutamic acid in response to osmotic stress is proposed to occur by a constitutive enzyme glutamine synthetase, that cyclizes the glutamate into pyroglutamic acid in the absence of ammonia.[25] This activity of PCA could be useful in controlling hyperosmolarity-induced mucosal damage.

Copper is an essential component of many biological systems and is an important cofactor for many enzymes.[26] High levels of copper are, however, toxic and, despite bacteria and other micro-organism have developed several mechanisms for dealing with excess metal, the redox properties of copper can also cause virus inactivation and cellular damage in yeasts and bacteria.

In vitro evaluation of virucidal effectiveness of a combination of PCA (4 percent) and CS (2 mg/100 mL) against human rhinovirus Type 14 was performed. Results showed that the combination had a strong virucidal effect and, after 15 minutes and after 180 minutes, it exerted a significant reduction (>4 log) of virus titration.[27]

More interestingly, PCA and CS combination (pirometaxine) improved significantly antimicrobial capacity against both Staphylococcus Aureus (SA) and Staphylococcus Epidermidis (SE) that are the most common pathogens of skin. These results, together with previous data on virucidal properties, highlight the role of PCA and CS as potent antimicrobial substances and remark their strong synergistic effect. Therefore, the combination of CS and PCA is an effective alternative to chlorhexidine and alcohol-based products in hand disinfection due to its virucidal and antimicrobial efficacy and to peculiar features such as immediate and residual activity and moisturizing effect.[28]

Aim of our observational study is evaluation of effectiveness of 5-Pyrrolidone-2-carboxylic acid (PCA) and copper sulphate pentahydrate (CS) association (pirometaxine) in the treatment of cold dry air rhinitis with or without viral/bacterial infection, exploiting their antiviral, antimicrobial and osmoprotectant effects. Our results showed that the majority of patients was healed within 7 days of treatment with PCA and CS. Twenty-seven patients (54%) reached a Total Symptoms Score of zero after 7 days and median number of days until healing was 6. After 7 days all patients had at least one-point reduction in nasal obstruction and rhinorrhea. Only 18% of cases had turbinate hypertrophy and 10% nasal mucosa hyperaemia after 7 days.

The main limit of this study is the absence of a control group. Further studies are necessary to better understand clinical potentialities of the combination of 5-Pyrrolidone-2-Carboxylic acid and copper sulphate in a self-limited disease, such as CDA-induced or viral rhinitis. To definitively demonstrate the effectiveness of therapy with PCA and CS, we need to plan randomized, prospective, double-blind controlled clinical trial with an homogenous population enrolled in the trial, in order to exclude every type of bias.

We demonstrated that PCA and CS (pirometaxine) represent a safe treatment with few side effects, like nasal burning

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and itch, that were reported by less than 10% of patients, without epistaxis. Treatment was interrupted in no case.

In conclusion, the association of 5-Pyrrolidone-2-carboxylic acid and copper sulphate (pirometaxine) represent a safe treatment for CDA-induced rhinitis with or without viral infection. Clinical importance of this therapy will be better understood with further studies, comparing results to those observed in a control group.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

PATIENTS CONSENT STATEMENT

All patients gave their written consent.

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