The clinical efficacy of potassium humate in the treatment of allergic rhinitis: A double blind placebo controlled trial

Justin J. Gandy, Johanna P. Meeding, Jacques R. Snyman and Constance E. Jansen van Rensburg*.

Department of Pharmacology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

*Correspondence to Dr C.E. Jansen van Rensburg

Department of Pharmacology,

Faculty of Health Sciences,

University of Pretoria,

PO Box 2034,

Pretoria,

0001,

South Africa,

FAX: +27-12-3192411

Tel: +27-12-3192622

Email: connie.medlen@up.ac.za

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Abstract

Background: The anti-inflammatory properties of products which contain high levels of humic acid such as peat, sapropeles and mumie is well described.

Objective: The aim of this study was to establish the clinical efficacy of brown coal derived potassium humate, since *in vivo* animal models suggested similar efficacy of brown coal derived humate to prednisolone.

Methods: In this double-blind, placebo controlled study, atopic volunteers were recruited and randomized to placebo or 1.8g potassium humate/day (3 divided dosages) when they presented with hay fever. The efficacy parameters used were; skin prick tests for wheal and flare reactions, nasal smears for inflammatory cell accumulation and cytokine levels. Patients also filled out daily symptom score cards to determine efficacy against symptoms of hay fever.

Results: Potassium humate resulted in a significant decrease in wheal and flare reactions as well as nasal eosinophil counts. These findings where supported by similar changes (not significant) in cytokine levels. Changes in symptom scores did not reach significance.

Conclusion: This proof of concept study clearly demonstrated potassium humate's potential in the treatment of inflammatory conditions such as hay fever.

Foot note: The corresponding author, CEJ van Rensburg, acts as consultant for the company.

Introduction

Although some anti-inflammatory properties of humate derived from peat, sapropeles and mumie have been described, no clinical studies has been done on the anti-inflammatory effects of humate derived from brown coal. Potassium humate compared favourably with prednisolone in suppressing contact hypersensitivity in a rat model [Van Rensburg et al., 2007].

It was found that potassium humate was safe at a dosage of 1000mg/kg body weight per day when administered to rats by gavage for one month [Van Rensburg et al., 2007]. Futhermore it was documented that 500mg potassium humate/kg body weight had any effect on pups after oral administration of the product to pregnant female rats. These results confirmed the results reported by the European Agency for the Evaluation of Medicinal Products (Feb 1999; http://www.emea.europa.eu/pdfs/vet/mrls/055499en.pdf). They also reported that the LD50 of humate in rats, after oral administration thereof, is greater than 11g/kg bw.

Allergic rhinitis is an inflammatory disease that is closely associated to other allergic conditions and may be viewed as a local manifestation of a systemic immune disorder [Holgate, 1999]. Successful therapy does not only suppress the signs and symptoms of local manifestations of atopic diseases but also to address the basic immune deregulation in order to establish a more efficient treatment. To date, treatments consist mainly of topical or systemic antihistamines, glucocorticosteroids, and decongestants. All these medicines are relatively expensive and are not without local or systemic side-effects and furthermore do not address the abnormal immune response as part of the allergic response.

The main aim of this study was to investigate the safety and anti allergic effects of brown coal derived potassium humate in patients suffering from exacerbations of hay fever during the grass pollen season in South Africa.

Methods used in this study to monitor the success of a treatment are the Total Sympton Score [Pradalier et al., 2007], the skin prick test [Grant et al., 1999] presence of eosinophils in nasal smears [Gleich et al., 1994], increase in serum cytokine levels associated with inflammation [Romagnani, 2000] and the state of activation of basophils [Falcone et al., 2006].

Methods and Materials

Treatment

Brown coal derived potassium humate was obtained from Unique Health Trust, Milnerton, South Africa. The product is marketed as a food supplement. Lactose was used as the placebo. All treatment capsules were uniform in size, colour, bottle filling, labelling, and packaging. Capsules contained 600mg of potassium humate or lactose. All subjects took one capsule of either potassium humate or lactose, three times daily. This study was approved by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria.

Safety Testing

The safety of brown coal derived potassium humate was determined by means of a full blood count, liver and kidney function tests namely sodium, potassium, creatinine, alkaline phosphatase, gamma GT, urea and urate. Blood samples were taken before and after the trial and were sent to Ampath laboratories, Pretoria, South Africa (SANAS Accredited Laboratory ISO-IEC 17025 medical testing laboratory # M0066) where the chemistry was done on a Roche Modular P800 and the haematology on a Beckman Coulter LH 750.

Patient selection criteria

A Total Symptom Score (TSS) questionnaire (Table 1) was used for selecting patients and to monitor success. The patients selected had to have a score of nasal symptoms equal to or

greater than 6 (≥ 6 over 12) on both days 48 hours preceding randomization. Except for allergic rhinitis, all patients selected were otherwise healthy. Participants were selected according to standardized inclusion and exclusion criteria, they could be male or female older than 12 years and had to have a positive skin prick test (> 3mm wheal). Participants were excluded if pregnant or breast feeding, any type of systemic disease other than allergic atopy, a washout period was required if participants were on any medication. Patents had to fill in a diary card on self assessment on a daily basis (Table 2). The study set out to attain 40 participants, 20 per arm.

Skin Prick Test

The allergen extract was introduced into the dermis which then resulted in an IgE-mediated response, and was characterized by a wheal and flare reaction. The test was performed on the inner aspect of the forearms, avoiding the flexures and the wrist areas. A lancet was used to prick the skin through the drop of allergen extract. The lancet was wiped with dry gauze between each prick in order to prevent a carry-over of allergens. The reactions occurred within 15 minutes after which the wheal was measured using a calliper. Positive and negative controls were also included in each series of tests.

A reaction of 3 mm greater than the negative control was regarded as positive. For each participant the skin prick test was done as part of the screening process and from the result the allergen the participant was most allergic to was then selected along with the positive histamine to be repeated at the end of the trial.

For each participant the skin prick test using multiple allergens, was done as part of the screening process and from the results the allergen the participant was most allergic to was then selected along with the positive histamine to be repeated at the end of the trial.

Eosinophil counts in nasal secretions

A nasal swab was plated out before and after the study. This smear was stained with eosin and methylene blue according to standard procedures and studied microscopically. A grading

scale (Table 3), as number of eosinophils present per 100 leukocytes, was used to monitor the response to treatment [Sheldon et al., 1967].

Basophils

Beckman Coulter Allergenicity Kit (Allergenicity Kit, Cellular Analysis of Allergy). The Allergenicity Kit consists of an optimized three-color combination of fluorescent monoclonal antibodies reagent, an activation solution, a positive control for IgE-mediated basophils activation, a stop solution, a lysing and a fixative solution. It is intended for "*In Vitro* Diagnostic Use" for the determination of activated basophils based on accurate basophils gating tool ($CRTH2^{pos}CD203c^{pos}CD3^{neg}$) performed on whole blood specimens (according to the manufacturer's instructions). The method was used according to the manufacture's instructions.

The cell population of interest was stained with monoclonal antibodies in the presence of the allergen or controls. The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. This procedure is carried out twice per patient eg. before and after treatment, the results are expressed as a percentage of activated basophils.

Cytokines

The FlowCytomix human Th1/Th2 10plex (hIFN-γ, hIL-1β, hIL-2, hIL-4, hIL-5, hIL-6, hIL-8, hIL-10, hTNF-α, hTNF-β) kit was used. It is a Multiplex Fluorescent Bead Immunoassay (FBI) for quantitative detection by Flow Cytometer of human Interferon-γ, Interleukin-1β, Interleukin-2, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-8, Interleukin-10, Tumor Necrosis Factor α and β in cell culture supernatants, serum, plasma, whole blood, or other body fluids. The method was used according to the manufacture's instructions.

The principle of the test is that microspheres are coated with antibodies specifically reacting with each of the analytes to be detected in the multiplex system. The beads are differentiated by their sizes and by their distinct spectral addresses. A mixture of coated beads for each analyte to be measured was incubated with the samples or standard mixture. The analytes present in the sample bind to the antibodies linked to the fluorescent beads.

A biotin-conjugated second antibody mixture is added, the specific antibodies bind to the analytes captured by the first antibodies. Phycoerythrin is added, binds to the biotin conjugate and emits fluorescent signals.

During the clinical trial serum from before and after the trial for each participant was frozen away in order to analyse the cytokines at the same time using the FBI.

Statistical Analysis

For all the statistics a statistician was consulted and the results were analysed using an ANOVA and adjusting for baseline on the STATA statistics software program.

Results

Patients

34 Patients successfully completed the trial, after randomization and dropouts; there were 15 in the placebo arm and 19 on the treated arm.

Total Symptom Score

When analyzing the data obtained from comparing the scores for the various times, no significant differences were observed when comparing the placebo group with that of the treated group (Table 4).

Skin Prick Test

With statistical analysis a significant difference was observed with a $p < 0.05$ when comparing the before and after results of the treated *vs* placebo groups respectively (Figure 1).

Eosinophil Count

With statistical analysis a significant difference was observed in the results expressed as an arbitrary number (between1-6) corresponding to the degree of eosinophils present on the smear when comparing the before and after results of the treated *vs* placebo groups respectively (Table 5).

Basophils

When analyzing the data using the difference between the positive and the test and then also the difference between the negative and the test, no significant differences were observed when comparing the placebo group with that of the treated group with respect to their before and after results (Table 6)

Cytokine levels

When analysing the data for both the pro and anti inflammatory cytokines no significant differences were observed when comparing the treated *vs* the placebo group (Table 7).

Discussion

In the clinical study the safety of brown coal derived potassium humate, at a daily dose of 1.8g, has been established, with no adverse events reported or observed. All haematology and biochemical parameters were within normal ranges and no statistically significant differences were detected. Although the total symptom score indicated no significant differences, a trend of decreasing scores was observed for both groups leading to the deduction that even though the trial was purposely run in and out of the pollen season, to take in account fluctuations in pollen count, in both groups' symptoms were alleviated.

When comparing the flare measurement it was demonstrated that there is a significant difference between the two groups, the treated groups flare measurements had significantly decreased, hereby demonstrating the anti-inflammatory potential. When comparing the smears of the two groups it was found that there is a significant difference between the two groups showing that the treated groups' nasal eosinophil recruitment had significantly decreased, which correlates with results obtained with other therapies for hay fever [Di Lorenzo et al., 2004]. No effect on basophil stimulation and thus histamine release was observed, and could therefore have a more specific anti-inflammatory effect.

With respect to IL-4 and IL-5 which cause influx of activated eosinophils and promotes IgE production respectively [Ronagnani, 2000], the mean of the placebo group increased whereas that of the treated group decreased, correlating with the results of nasal eosinophil result. However this increase was not significant. The small study size, intra and inter individual variability of cytokines make definite conclusions impossible.

The involvement of eosinophils is the trademark of allergic rhinitis. The accumulations of these cells to local tissue sites include the effects of cytokines and other cellular and humoral mediators [Linden et al., 1999]. Eosinophils are rarely present within nasal mucosa of individuals who are non-atopic and have no nasal symptoms. The recruitment and activation

of eosinophils is considered critical to clinical disease expression, through the ability of these cells, when activated, to release mediators that induce development of inflammation. So in decreasing the recruitment of eosinophils it proves the anti-allergic effect of potassium humate on yet another marker of allergic rhinitis.

A T helper (Th) 1/Th2 cytokine imbalance with a predominance of Th2 cytokines has been suggested of pathogenic importance in allergic rhinitis [Ronagnani, 2000]. Th2 cells fulfil an important role in humoral and allergic immune responses, especially through the production of IL-4, IL-5 and IL-10. Th2 cells can be readily found in the late-phase response in the lung and after intradermal injection of antigens into the skin. Some of the cytokines secreted by these cells have direct pro-inflammatory effects; both IL-4 and IL-5 promote the recruitment and survival of eosinophils and mast cells [Romagna, 1997; Mosman et al.,1986; Cherwinski et al., 1987; Mosmann et al., 1989; Mosman et al., 1996;].

According to results of Van Rensburg et al., (2009) the anti-inflammatory properties of potassium humate could partially be ascribed to the inhibition of the complement cascade and a decrease in the production of the inflammatory cytokines TNF-α, IL-1β and IL-6 [Van Rensburg et al., 2009]. Jooné *et al* described results obtained with oxihumate, a water-soluble humate obtained through a wet oxidation of bituminous coal, decreases the expression of complement receptor 3 (CR3) by stimulated human neutrophils as well as the adhesion properties of these cells [Jooné and van Rensburg, 2004]. The complement system is an important connection between adaptive and innate immunity and researchers have identified certain anaphylatoxins that may be effectors in rhinitis, thus the activation of complement may be synergistically linked to IgE mediated responses, and thus an inhibition of this may lead to potential therapy for allergy sufferers [Gerhard and Gerhard, 2002].

In conclusion: Potassium humate clearly demonstrates the potential as an anti inflammatory product in the treatment of atopic conditions such as allergic rhinitis as demonstrated by suppression of eosinophils. The fact that the symptom score reduction did not reach significance can be ascribed to the relative small sample size, the change over of the season

and the duration of treatment. This product needs to be further investigated in this disease area.

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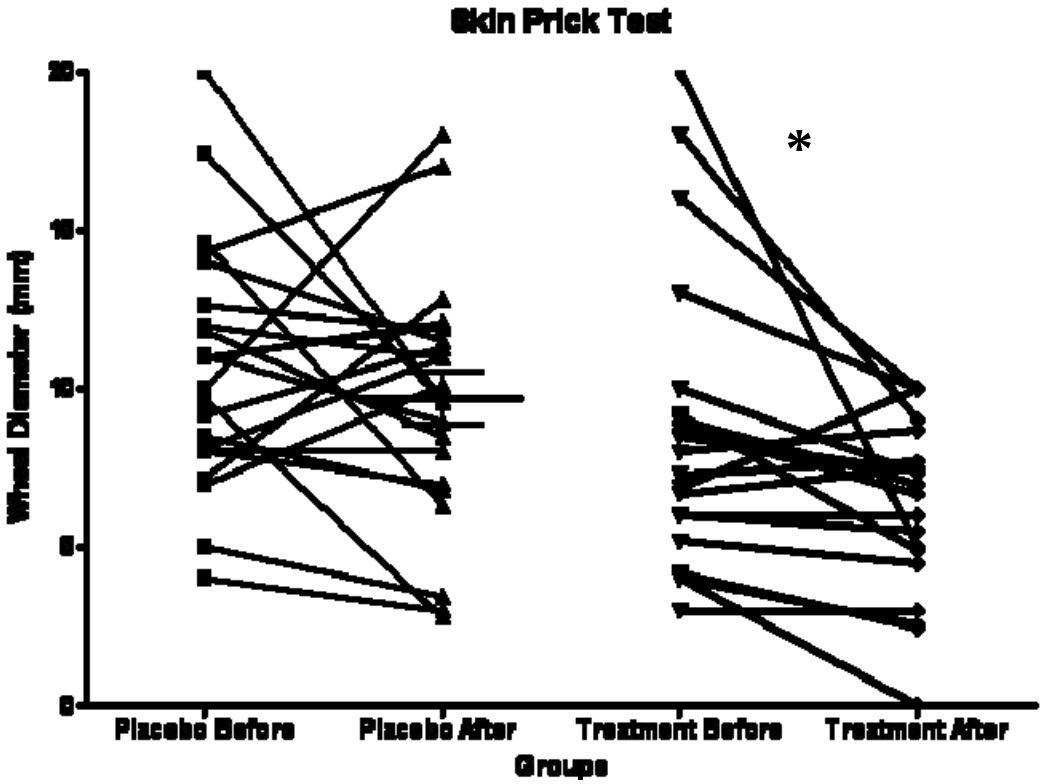
Legend to figure:

Fig. 1. Measurements of wheal (diameter in mm) before and after the study of the treated *vs* placebo groups with respect to their skin prick tests.



* Indicating statistical significance ($p < 0.05$)

Fig. 1

Table 1. Total Symptom Score

Symptom assessment	
Nasal symptoms	**Non-nasal symptoms**
Sneezing	Ocular itching (burning scratching etc.)
Running nose	Tearing of eyes
Nasal itching	Itching of ears/and palate
Nasal blockage	Ocular redness (conjunctival injection)

Table 2. Dairy card

Score	**Symptom**
0	No symptoms present
1	Mild symptoms occasionally present but not troublesome
2	Moderate symptoms, frequently present and annoying
3	Severe symptoms continuously present and interfering with work or sleep or other daily activity eg. Sport.

Table 3. Grading used to estimate eosinophil numbers on nasal swab smears.

	First 48 Hours		1st Mid 48 Hours		2nd Mid 48 Hours		3rd Mid 48 Hours		Last 48 Hours	
	Morning	**Evening**	**Morning**	**Evening**	**Morning**	**Evening**	**Morning**	**Evening**	**Morning**	**Evening**
Treated	**30.8**	**33.7**	**29.8**	**31.1**	**26.2**	**26.7**	**26.7**	**26.8**	**26.4**	**28.6**
Placebo	**30.1**	**34.5**	**28.5**	**31.3**	**24.7**	**28.3**	**22.6**	**25.7**	**22**	**24.2**

Grade	**Eosinophils**
1	No cells seen
2	Only occasional eosinophils on smear.
3	Present but scanty and scattered throughout smear.
4	Approximately 15-30 cells
5	Approximately 30-75 cells
6	Approximately > 75 cells

Table 4. Total symptom scores before and after the study of the treated *vs* placebo groups.

Morning: Average score for 2 mornings.

Evening: Average score for 2 evenings.

First 48 Hours: Average scores of first 2 days of the trial.

1st Mid 48 Hours: Average scores of days 7 and 8 of the trial.

2nd Mid 48 Hours: Average scores of days 14 and 15 of the trial.

3rd Mid 48 Hours: Average scores of days 21 and 22 of the trial.

Last 48 Hours: Average scores of last 2 days of the trial.

Table 5. Degree of eosinophil presence (using the grading system) in smears obtained from the treated *vs* placebo groups.

	Degree of eosinophil presence			
	Before		**After**	
	Mean	**SD**	**Mean**	**SD**
Treated	**2.63**	**1.12**	***1.84**	**0.60**
Placebo	**2.80**	**0.83**	**2.95**	**0.76**

* Indicating statistical significance ($p < 0.05$)

Table 6. Placebo *vs* treated groups with respect to the difference in percentage of activated basophils before and after treatment.

	Visit 2			**Visit 4**		
	% Activated Basophils			**% Activated Basophils**		
Placebo	**Positive**	**Negative**	**Test**	**Positive**	**Negative**	**Test**
Average	**42.05**	**8.05**	**50.71**	**43.79**	**8.32**	**49.05**
SD	**27.33**	**11.22**	**31.89**	**24.57**	**15.11**	**29.26**
Treated						
Average	**40.75**	**5.06**	**41.22**	**38.87**	**8.63**	**47.53**
SD	**25.2**	**7.36**	**19.83**	**25.94**	**17.42**	**26.51**

Table 7. Average means and standard deviations of the Th1 and Th2 cytokines before and after the trial respectively.

	Cytokine levels (pg/ml)							
Cytokines	**IL-1B**		**IL-8**		**IL-4**		**IL-5**	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Placebo								
Average	**21.93**	**29.77**	**96.66**	**126.23**	**450.94**	**455.98**	**59.79**	**110.97**
SD	**9.88**	**23.5**	**51.67**	**89.13**	**1847.11**	**1397.49**	**54.04**	**176.98**
Treated								
Average	**57.69**	**52.65**	**30.23**	**26.28**	**119.91**	**110.94**	**19.05**	**14.58**
SD	**75.94**	**55.65**	**28.47**	**26.62**	**78.65**	**110.98**	**30.76**	**16.57**