

The effect of corona discharge on Der p 1

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Summary

Background Reduction of house dust mite allergens in the domestic environment can play an important part in reducing sensitization and in the amelioration of symptoms in atopic individuals. Chemical and physical methods have been tried with varied levels of success. The present paper presents a novel electrostatic way of destroying Der p 1, the major mite allergen.

Objective To assess the efficacy of negative Trichel, negative continuous glow, positive pulse and positive continuous glow corona in destroying Der p 1. To determine whether ozone has any effect on the integrity of Der p 1 in the experimental conditions present.

Methods A simple point-to-plane apparatus was used to irradiate samples of Der p 1 for periods of 1, 15, 30, 45, 60, 120, 180, 240 and 300 min. Controls were exposed to the atmosphere with no corona products present for the equivalent time. The effect of the corona by-product ozone was investigated alone by exposing samples of Der p 1 to molecular ozone for 60 min. Der p 1 concentration was quantified by two-site monoclonal antibody ELISA.

Results High current negative glow resulted in a 67.37% reduction in Der p 1 concentration after 300 min compared with a 50.5% reduction from a low current Trichel regime. High current positive glow corona gave a reduction of 25.22% while a low current positive pulse corona caused a 13.72% reduction after 300 min. All these reductions were statistically significant ($P < 0.05$) compared with unexposed controls. Negative corona always gave greater percentage reductions in Der p 1 concentration for each time exposure investigated. The pattern of percentage reduction follows an exponential rise to maximum relationship in respect to time. Samples of Der p 1 were not affected by exposure to molecular ozone.

Conclusion These data indicate corona products to be a powerful new method of destroying Der p 1 allergen that is not dependent on the presence of the oxidizing corona product ozone.

Keywords Der p 1, corona discharge, ozone, house dust mites

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Introduction

Over the past 20–30 years the incidence of bronchial asthma has steadily increased in almost every country around the globe [1]. In parallel to this there have been reports of up to fivefold increases in the prevalence of house dust mites (HDMs) in random samples collected over a 10-year period in both temperate [2] and warmer [3] climates. As the link between HDM occurrence and atopic asthma, rhinitis and eczema is now well established [4], methods of reducing exposure to HDM allergen must be important in the avoidance and control of atopic disease. However, attempts over the last four decades to eradicate HDMs from the typical domestic environment have been largely unsuccessful.

Many methods exist to minimize contact with HDMs. These include both physical, such as employing extremes of temperature

and relative humidity (RH), and chemical methods such as the use of acaricides to kill the mites and chemicals to destroy the HDM allergens (see [5] for a review). In this paper a novel way of destroying the major HDM (*Dermatophagoides pteronyssinus*) allergen Der p 1 using the electrical phenomenon corona discharge is described.

Corona discharge occurs when a high voltage is applied between electrodes in a non-uniform geometry, e.g. when one electrode has a small radius of curvature (i.e. a point) and the other is a plane [6]. This non-uniform electrode geometry leads to electric field intensification such that the local breakdown potential of the surrounding atmosphere is exceeded. The high-field region near the point is characterized by the generation of uni-polar ions of the same polarity as the point electrode. The ions will be extracted from the corona discharge and will migrate under the influence of the electric field to the opposite electrode. In this way, a stream of uni-polar ions may be created and directed towards a surface. This technique is widely used in industrial applications such as electrostatic powder coating [7], electrocopying machines [8] and electrostatic particulate precipitation [9]. In addition to these ions, heat, both molecular and ionic ozone and chemically potent

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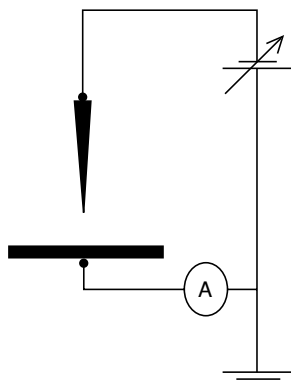


Fig. 1. Schematic diagram of the point-to-plane corona apparatus.

excited atoms and molecules called neutral metastable species are produced.

Corona discharge is a generic term for a spectrum of different types of corona. For negative corona in air, Trichel pulses of current are first to appear. As the electric field is enhanced, a continuous glow corona regime is reached [10]. Positive coronas have analogous regimes called positive pulse and positive continuous glow. This paper describes the effect of these different types of corona on samples of evaporated aqueous Der p 1 solution using a point-to-plane corona arrangement. The effect of molecular ozone was also investigated to determine whether this oxidizing agent was responsible for the destruction of Der p 1.

Materials and methods

Preparation of the evaporated aqueous Der p 1 samples

Cultures of HDMs were established by using material obtained from domestic vacuum cleaner bags that tested positive for Der p 1. Cultures were maintained on a substrate of dog biscuit and dried yeast mixed in a ratio of approximately 30 : 1 [11]. The established HDM culture was sieved to below 63 μm . An aqueous solution of this HDM culture was prepared by adding 15 g to 400 mL of distilled water. This mixture was stirred well to allow the Der p 1 to dissolve. The mixture was then passed through filter paper (Whatman, qualitative, grade 4) to remove solid material. Thimerosal (sodium ethylmercurithiosalicylate) was added to this solution at a concentration of 0.001% to prevent bacterial, fungal and mould growth.

Samples were prepared by pipetting 100 μL of this Der p 1 solution onto 20 mm² squares of aluminium foil. The sample and its paired control were prepared adjacently. These were then dried at 37 °C.

Exposure of Der p 1 samples to corona discharge

A simple point-to-plane corona apparatus was made using a pin with point radius of 45 μm fixed with an interelectrode gap of 15 mm above a square, planar 20 mm² aluminium electrode. This is illustrated schematically in Fig. 1. Onto this a Der p 1 sample was placed. The pin was connected to a d.c. high voltage supply of a chosen polarity to achieve the desired corona regime. For negative Trichel, ionic bombardment equivalent

to a corona current of 25 μA was used, for negative continuous glow the current was 90 μA . For positive pulse the current was 15 μA , and 5 μA for positive continuous glow. These values were chosen as being representative of the desired type of corona generated at a constant relative humidity of 45%. The corona current was maintained at a constant value throughout each test.

Samples were exposed for 1, 15, 30, 45, 60, 120, 240 or 300 min. The sample was then removed and stored for subsequent assay to determine the concentration of Der p 1. The control Der p 1 sample was exposed to the ambient atmosphere for each test period, and then also stored for later assessment. The results were expressed as a mean percentage reduction in Der p 1 concentration of the samples compared to their paired control. Six replicates were completed for each corona regime.

All samples were analysed for Der p 1 concentration by a two-site monoclonal antibody ELISA (INDOOR Biotechnologies Ltd, Cardiff, UK).

Exposure of Der p 1 samples to molecular ozone

An ozone production unit (STRG-PC-U-tube, Starna Ind Ltd, Essex, UK) was used to generate ozone in an enclosed box connected to a compressed air supply. The ozone was blown out of the box onto a Der p 1 sample, the concentration being determined by the airflow. Ozone concentration was measured using Dräger short-term ozone detecting tubes. Concentrations of 15 ppm, 50 ppm and 110 ppm were investigated. Typical ozone concentrations emitted during corona discharge with one pin were approximately 0.5–1 ppm. These higher concentrations were chosen to clearly show whether ozone had any effect on Der p 1. Following a period of 5 min, in which the concentration of ozone was allowed to stabilize, the Der p 1 sample was exposed for 1 h. Control samples were exposed to the corresponding airflow without the ozone production unit in operation. The RH of the airflow was constant at 10%, and six replicates were completed for each ozone concentration.

Statistical analysis

For each length of exposure, the Der p 1 concentrations of the six exposed samples were compared to their paired controls by the *F*-test. The concentration data for each time period was then analysed using the Paired *t*-test for means, or the Mann–Whitney *U*-test where appropriate. These data were then presented as mean percentage reductions in Der p 1 concentration calculated by comparing the Der p 1 sample to its equivalent control in the specific time period. The Mann–Whitney *U*-test was also used to compare the percentage reductions caused by the intrapolarity corona regimes at each time period. Statistical significance was defined as $P < 0.05$.

Results

The effect of corona regimes on Der p 1

All the corona regimes studied resulted in a reduction in Der p 1 concentration. Figure 2 shows that negative corona regimes reduced the concentration of Der p 1 in an exponential growth to maximum relationship with respect to time. Both regimes fit the non-linear trend line (exponential rise to maximum) well with R^2 values of 0.893 and 0.895 for Trichel and continuous

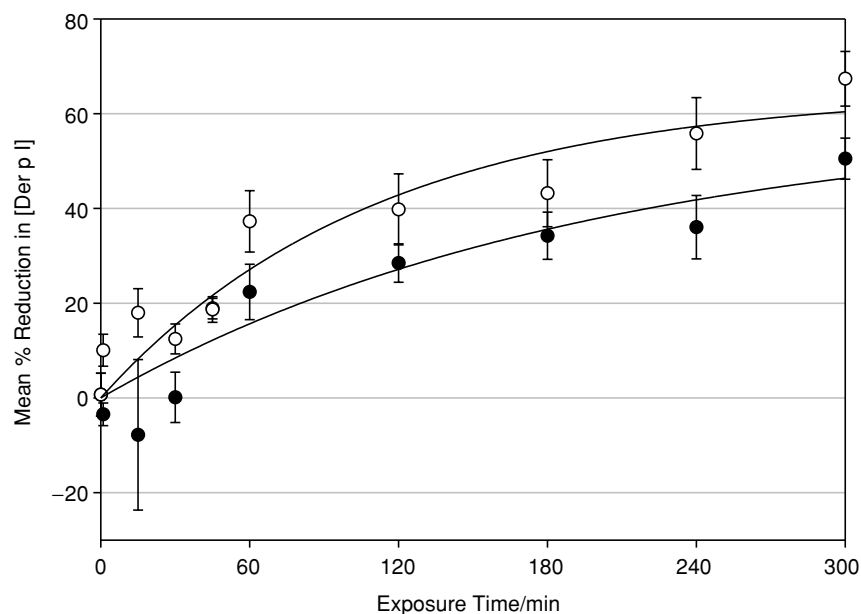


Fig. 2. The percentage reduction in Der p 1 concentration after irradiation by negative continuous glow (○), or negative Trichel (●) corona regimes. (Values are the mean of six replicates, standard error of mean (SEM) is shown).

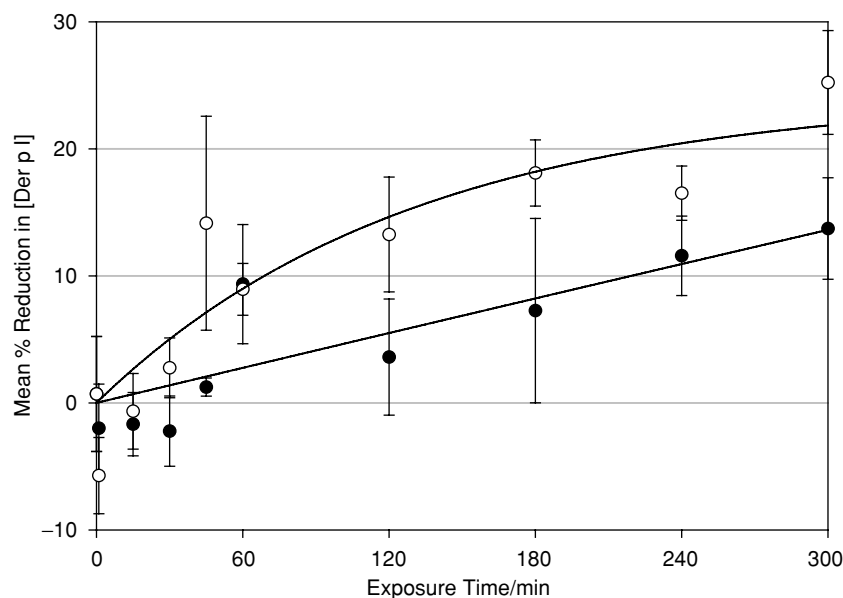


Fig. 3. The percentage reduction in Der p 1 concentration after irradiation by positive continuous glow (○), or positive pulse (●) corona regimes. (Values are the mean of six replicates, SEM is shown).

glow, respectively. Negative continuous glow corona showed a greater efficacy for destroying Der p 1 than Trichel, with the percentage reduction reaching 67.37% compared with 50.5% for Trichel after 300 min. When the concentration of the samples was directly compared to their paired control, the reductions with Trichel corona (25 μ A corona current) after 45, 180, 240 and 300 min exposure were significant ($P < 0.05$). Conversely, with a negative glow regime, all reductions were statistically significant.

Figure 3 shows the percentage reductions following exposure to positive corona regimes. Only the reduction in concentration after exposure times of 240 and 300 min were significant for positive pulse corona (5 μ A). However, the higher energy positive continuous glow corona (15 μ A) resulted in significant

reductions after 60, 120, 240 and 300 min. The relationship between the exposure time of a sample and the percentage reduction in the Der p 1 concentration also appears to be exponential ($R^2 = 0.771, 0.850$ for positive pulse and glow, respectively) with positive glow reaching a maximum of 25.22% in the exposure times measured. After 300 min positive pulse gave a 13.72% reduction.

If the percentage reductions obtained by the two intrapolarity regimes after a specific period of exposure are compared, only the reductions with negative Trichel and negative glow after 300 min are significantly different ($P = 0.04$). This means that overall there is no statistical difference in the ability of the high and low energy corona of the same polarity to reduce Der p 1 concentration. As the two regimes within the same polarity are

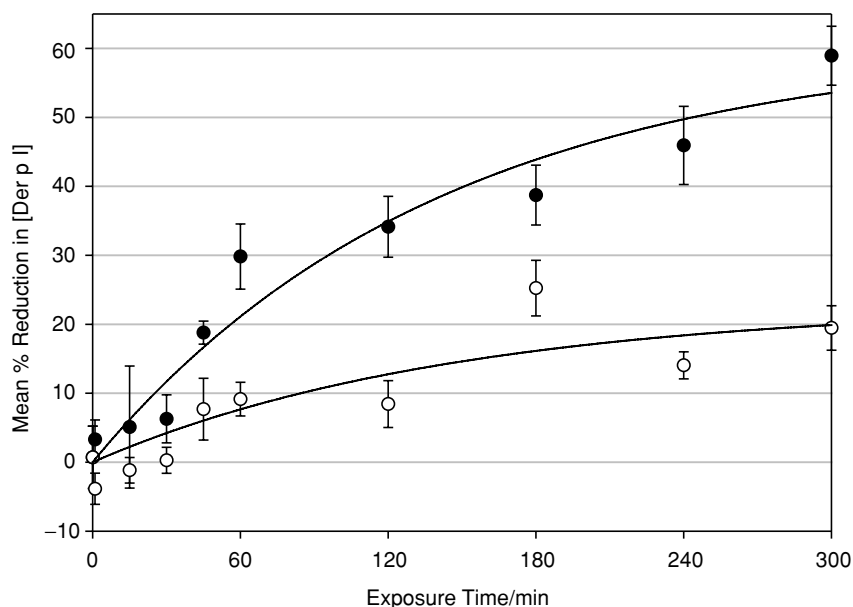


Fig. 4. The percentage reduction in Der p 1 concentration after irradiation by negative (●), or positive (○) coronas. (Values are the mean of twelve replicates, SEM is shown).

statistically non-different, the data from both can be combined. Figure 4 shows the exponential relationship between the time of exposure to either negative or positive corona and the percentage reduction of Der p 1 concentration. It can be seen that Der p 1 concentration was reduced more effectively by negative corona for each time exposure reaching a maximum of 58.94% after 300 min compared with only 19.47% by positive corona.

The effect of ozone on Der p 1

There was no difference in the concentration of Der p 1 between samples exposed to varying levels of ozone concentration produced by the ozone production unit and unexposed controls.

Discussion

This study indicates that exposure to either negative or positive corona regimes results in the reduction of Der p 1 concentration. Negative corona types showed a greater efficacy at destroying the Der p 1 with a mean percentage reduction 42.15% higher than the greatest positive corona reduction. For both polarities, the higher current continuous glow regimes gave a greater mean reduction in Der p 1, although the efficacies of these intrapolarity regimes were not statistically significant. Ozone was shown not to cause any reduction in Der p 1 concentration under these experimental conditions. However, many other chemical species are present in a corona discharge and these may contribute to the reductions measured.

These experiments do not indicate whether the Der p 1 molecule is being chemically modified, cleaved or denatured in the true sense of the term. True denaturing results in the alteration of a protein's tertiary (and/or quaternary) structure with no chemical changes. The ELISA results indicate an alteration in the epitopes, which prevents the monoclonal antibody from binding. Although the mechanism of this change is not known, any difference in the molecule would affect its allergenicity. Der p 1 is a cysteine protease and its immunogenicity is

thought to be reliant on its functionality [12], any alteration to its structure could adversely affect its function/allergenicity. It is also possible that interference between the gaseous ions and the electrical characteristics of the protein is causing a change in its structure.

Further work would be required to identify the mechanism by which corona destroys Der p 1, and whether other proteins and allergens are similarly affected. This preliminary investigation has confirmed that exposure of Der p 1 to either negative or positive corona products leads to a reduction in Der p 1 concentration. The corona generators could be optimized to produce a greater reduction in allergen and since the corona discharge is created at ambient temperature and air pressure, the ion generation method lends itself to applications in domestic appliances such as vacuum cleaners, air conditioners and ionisers. Although complete denaturing was not achieved over an exposure period of 300 min, it is likely that application of the technology in electrical appliances would be more appropriate where continuous, or semicontinuous, operation is envisaged.

Any reduction in the concentration of Der p 1 is of potential benefit within the domestic environment. The use of this technology within the home could keep the allergen levels below the minimum needed to elicit sensitization and symptoms i.e. below 2 µg/g of dust [13]. Another benefit to this method of cleaning the home of allergens is its acaricidal properties. Although not discussed in this paper, unpublished results suggest that mites do not survive exposure to corona products. This is not surprising, as ozone has been reported to kill a number of organisms including insects [14]. Ionisers utilizing corona discharge are also known to reduce the level of airborne particulates [15]. This would also reduce the amount of allergen breathed into the body.

Acknowledgements

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