In vivo models of occupational asthma due to low molecular weight chemicals

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Abstract
The aim was to review the development of in vivo models of asthma due to low molecular weight chemicals, in particular, those aspects that may be important to the understanding of occupational asthma in humans.

Keywords: chemically induced asthma; respiratory hypersensitivity; disease models

Occupational asthma is now the most prevalent occupational lung disease in many western countries. There are over 500 new cases reported a year in the United Kingdom, and there are now over 250 compounds reported as potential causes of occupational asthma. There has been increasing interest in the past few years in the possible mechanisms by which low molecular weight chemicals may induce asthma in humans. It has not been possible to detect specific immunological responses to many of these compounds although the clinical profile of asthma is clear. The associated increase in airway responsiveness and the underlying airway inflammation found in patients with occupational asthma also suggest that the underlying mechanism is that of an acquired hypersensitivity response to sensitising agents. There is little known about the factors and mechanisms important in sensitisation and airway responses induced by exposure to these compounds in humans. To attempt to answer some of these questions, there have been several attempts to develop animal models of occupational asthma. The development of these models and their role in improving our understanding of chemically induced asthma is described here.

Four groups of chemicals have been mainly studied to date—namely, the isocyanates, acid anhydrides, platinum salts, and plicatic acid.

Isocyanates
The isocyanates form a group of highly reactive compounds that are widely used in the paint and printing industry and in the production of polyurethane foam. They are now the leading cause of occupational asthma in the western world. There have been many attempts by several groups to develop models of sensitisation to isocyanates. Scheel et al as early as 1964 showed that injections of an isocyanate conjugated to a protein induced specific immune responses in rabbits. In 1979 Tse et al injected mice with a protein conjugate of toluene diisocyanate (TDI) and was able to induce homocytotropic reaginic antibodies that were hapten specific. With this technique Chen and Bernstein et al found that immunoglobulin G and E (IgG and IgE) antibodies induced by isocyanate protein conjugates in two breeds of guinea pig elicited immunological responses that were specific for the hapten whereas precipitating antibody responses were directed against new antigenic determinants formed on the hapten protein molecule. They also found with whole body plethysmography that passive transfer of serum samples from sensitised to naive animals resulted in a significant pulmonary response when they were challenged intravenously with the immunising antigen. These studies have been useful in showing the potential of isocyanates, albeit in the form of a protein conjugate to induce both a specific immune response and a pulmonary response. Patterson et al successively sensitised dogs through an endotracheal tube to TDI and diphenylmethane diisocyanate (MDI) alone. Further exposure to the sensitising chemical provoked increases in pulmonary resistance.

Karol et al exposed only the heads of guinea pigs to 0.25 ppm of TDI or p tolyl isocyanate for three hours on five consecutive days, which resulted in hapten specific immunological responses to a protein conjugate of the isocyanate. Specific increases in respiratory rates were noted on challenge with the protein conjugate. In a further study they exposed different groups of animals to varying schedules of exposure but the same cumulative dose of TDI and found that there was a threshold of exposure below which sensitisation did not occur. A moderately high exposure (600 ppb) was required to induce sensitisation. The pattern of exposure was critical for sensitisation. Re-exposure to TDI alone provoked specific respiratory responses in sensitised animals. These respiratory responses were also found to be concentration dependent. These data have recently been confirmed by Aoyama et al.

The mechanism by which TDI might induce an immunological response has also received attention. Absorption of inhaled radiolabelled TDI into the blood stream is concentration dependent in guinea pigs even at levels of exposure below the threshold limit value (TLV) and induces conformational change in the albumin enriched fraction of guinea pig.
serum samples predominantly affecting the alpha helix.\textsuperscript{13} Inhaled TDI binds to several proteins of varying sizes in the bronchoalveolar fluid of guinea pigs; the most prominent protein is considered to be albumin.\textsuperscript{14}

Although inhalation exposure is considered to be the preferred method of sensitisation, it has been shown that sensitisation by intradermal injection or topical application may be a more effective method than inhalation at inducing both immunological and pulmonary responses to MDI in guinea pigs.\textsuperscript{15} It is of interest to note that previous inhalation of TDI\textsuperscript{16} or MDI\textsuperscript{17} downregulates the effect of systemic sensitisation on contact sensitivity and IgE immune responses in the guinea pig. Recently nasal application of TDI has been used to induce sensitisation\textsuperscript{18} and has been found to be associated with increased turnover of histamine in both nasal and lung mucosa.\textsuperscript{19} With this method of sensitisation, Sugawara \textit{et al} provoked changes in pulmonary responses that occurred both immediately and again 10 hours after nasal challenge to TDI.\textsuperscript{20} Histological examination showed muscle hypertrophy, mucin hypersecretion, and a predominantly eosinophilic infiltrate of the peribronchial area.

Sheppard \textit{et al} published a series of papers that examined the effect of acute single exposure to TDI in guinea pigs on airway reactivity and airway inflammation.\textsuperscript{21–24} The authors found that exposure to TDI for one hour at a concentration of 2 ppm induced an increase in airway reactivity and the number of polymorphonuclear leucocytes in the airways of guinea pigs studied six hours after exposure. They subsequently showed in animals pretreated with capsaicin (to deplete sensory nerves of neuropeptides) that tachykinins played an important part in this response. Cibulas \textit{et al} similarly found that depletion of granulocytes did not inhibit airway hyperreactivity induced by TDI in guinea pigs.\textsuperscript{25} Although it is difficult to know what relevance these studies might have to asthma induced by TDI, the findings suggest that an increase in airway reactivity and airway inflammation could predate sensitisation and the development of asthmatic symptoms. More recently, Erjefalt and Persson reported a dose dependent increase in plasma exudation in the airways of guinea pigs after TDI was placed directly on to the trachea\textsuperscript{26} and Mapp \textit{et al} have found that TDI can induce smooth muscle contraction in the rat bladder, an action possibly through the cyclo-oxygenase pathway.\textsuperscript{27} This research, although of considerable interest, does not provide us with a model of TDI asthma as we currently understand it but may permit speculation on the immunological and non-immunological mechanisms important to the development of isocyanate induced asthma.

In humans although the clinical profile of isocyanate induced asthma is consistent with an immunological process, only a portion of people with isocyanate induced asthma show specific immune responses. There has been no difference found in the histopathological changes in those with extrinsic, intrinsic, or TDI induced asthma.\textsuperscript{28} In a recent study genetic factors that influence immunological responses were found to be important to the development of TDI asthma.\textsuperscript{29} By contrast, Karol \textit{et al}\textsuperscript{30} report that specific IgE antibodies to TDI may be associated with early but not late asthmatic responses on inhalation challenge. Late asthmatic responses were more likely to occur in those subjects who showed airway hyperresponsiveness.

**Platinum salts**

Early work on the development of an animal model of sensitisation to platinum salts failed to induce an immunological response to the free salt but did so to the protein conjugate of the salt.\textsuperscript{31} These results were surprising given that platinum salts are generally considered to be potent sensitising agents in humans. Biagini \textit{et al} postulated that workers employed in platinum refineries are exposed not only to platinum salts but also to acidic mists and irritant gases that may affect sensitisation to the platinum salts.\textsuperscript{32} They showed in cyanomolgous monkeys that exposure to inhaled hexachloroplatinate in combination with ozone was much more likely to induce a positive skin test to platinum salts and increased airway responses to both aerosolised hexachloroplatinate and methacholine.\textsuperscript{32} This study clearly suggested that cofactors such as irritant gases might be important in the development of sensitisation and respiratory symptoms to occupational agents. In platinum refinery workers, a strong association has been noted between airway hyperresponsiveness to cold air and conversion to a positive skin prick test to platinum one year later.\textsuperscript{33}

**Plicatic acid**

The desire to develop models of sensitisation to plicatic acid resulted from data that suggested that although asthma due to plicatic acid was due to an acquired hypersensitivity it was only associated with a specific IgE immune response in 40\% of patients with asthma. Chan \textit{et al} were able to sensitise rabbits by injecting plicatic acid conjugated with ovalbumin into the peritoneal space but only five of 19 rabbits developed specific IgE antibodies to plicatic acid.\textsuperscript{34} Exposure to the conjugate by intravenous injection but not by aerosol provoked acute airway responses. More recently Salari \textit{et al} have shown that in guinea pigs sensitised to the conjugate, challenge with plicatic acid alone provoked tracheal contraction and release of histamine, prostaglandin D2, and leukotriene D4 from both lung mast cells and blood basophils.\textsuperscript{35} The magnitude of these responses was considerably less than that found when ovalbumin alone was used, which indicates that plicatic acid is a less potent sensitisier than ovalbumin. Similarly, histamine and leukotriene release has been reported in patients with asthma induced by plicatic acid during inhalation challenge.\textsuperscript{36}
Acid anhydrides

Interest in the capacity of the acid anhydrides to induce sensitisation began soon after the initial reports in 1937 of asthma due to phthalic anhydride. As early as 1940 Jacobs et al injected guinea pigs with an acid anhydride in olive oil and were able to provoke immediate skin responses to the same anhydride two weeks later. Landsteiner and Chase confirmed this finding with the serum samples of sensitised guinea pigs and showed passive anaphylaxis that could be diminished by preheating. In the mid-1970s, it was recognised that exposure to the acid anhydrides in the workplace could induce several different conditions in humans. This was followed by several experiments that examined the immunological and pulmonary responses on exposure to trimellitic anhydride, by varying routes, in several species. Exposure to trimellitic anhydride (TMA) by tracheal installation or by inhalation induced specific immunological responses, and repeated exposure by inhalation was used to develop a rat model of acid anhydride induced pulmonary haemorrhage. In recent years Botham et al exposed only the noses of guinea pigs to TMA dust for three hours on five consecutive days and induced a specific IgE immune response to TMA conjugated to guinea pig albumin (TMA-GPSA) in a few exposed guinea pigs. Subsequent intradermal sensitisation increased the number of guinea pigs that developed specific IgE responses. With these models of sensitisation, they exposed sensitised guinea pigs to further doses of inhaled TMA dust and found an increase in respiratory rate in sensitised but not in control guinea pigs. Unfortunately, there was no more detailed information on the nature of the pulmonary response. The same group explored the immunological response to TMA in this model further and found that systemic challenge with alum precipitated hapten protein conjugate in guinea pigs sensitised by inhalation down regulated the IgE immune response to TMA-GPSA and reduced the pulmonary response to subsequent exposure by inhalation. The authors speculated that this could be due to IgE iso-type specific suppressor cells that may have a protective role in the healthy lung by preventing sensitisation similar to that found with isocyanates. In another experiment they compared the effect of topical TMA and topical dimethyldichlorotetrahydrobenzanthrone (DCNB, known to cause contact dermatitis) on sensitisation in mice. They found that TMA induced IgG2b antibodies but DCNB induced IgG2a antibodies. This suggested that the induction of differing subsets of T helper cells may account for the differing clinical manifestation of sensitisation to these compounds in humans. Similarly TMA, but not DCNB, induced specific sensitisation of peritoneal mast cells in mice. Recently, with a similar method of intradermal activation with TMA in corn oil, a guinea pig model of sensitisation was developed that is suitable for assessment of airway responses to both TMA-GPSA and TMA dust. Initially it was found that intradermal sensitisation with TMA induces a specific immune response to TMA-GPSA and not to TMA alone, which is similar to the response found in humans. The method of sensitisation was optimised to give the maximal increase in lung resistance to intravenous TMA-GPSA. Instilling TMA-GPSA on to the trachea of sensitised guinea pigs was found to be an effective method of examining immediate airway responses. These responses were associated with airway microvascular leakage predominantly in the post-capillary venules of sensitised animals. The response was of an order that allowed investigation of the underlying mechanism. The increase in lung resistance could be significantly inhibited by an antihistamine (chlorpheniramine), a platelet activating factor inhibitor (WEB 2086), and the non-steroidal anti-inflammatory nedocromil sodium, but was not significantly inhibited by a lipoxygenase inhibitor (BW 4AC). The increase in airway microvascular leakage was only inhibited by chlorpheniramine. Treatment with cyclosporin during sensitisation significantly attenuated both the increase in lung resistance and airway microvascular leakage in this model. In a separate experiment, a significant increase in mucous secretion in sensitised animals exposed to TMA-GPSA was noted. Although the associated bronchoconstriction was again inhibited by an antihistamine, it did not affect the degree of mucous secretion, which was only significantly inhibited by combining pre-treatment of capsaicin with the ganglion blocker hexamethonium. This suggests that local axon reflexes may be important in the control of mucous secretion in this model. Although mucous secretion did not seem to be very important in the acute bronchoconstrictor response in this model, it suggests that hypersecretion of mucous may occur in patients with asthma due to low molecular weight chemicals.

Airway hyperresponsiveness is a feature of symptomatic patients with occupational asthma and transient increase in airway responsiveness has been shown to precede the development of late asthmatic responses to low molecular weight chemicals in patients undergoing specific bronchial provocation tests to these agents. A mean twofold increase in airway responsiveness has been shown in sensitised guinea pigs exposed to TMA dust. This response is maximal eight hours after exposure and is associated with a significant increase in the number of submucosal eosinophils. Although this might suggest that the increase in airway responsiveness was due to the increase in the number of submucosal eosinophils, there was no correlation between the number of eosinophils and airway responsiveness in the groups of guinea pigs assessed at 2, 8, and 24 hours after exposure. This suggests that the level of eosinophilic activation might be a more important factor in the development of increased airway responsiveness than the total number of eosinophils.
number of eosinophils in the airways. In a subsequent experiment it was found that repeated doses of inhaled budesonide (a topical steroid) could inhibit the increase in airway responsiveness but not the associated increase in eosinophils in the submucosa or bronchoalveolar lavage fluid. A greater dose of budesonide might have had a greater effect on airway inflammation. This response may be similar to that found in patients undergoing specific inhalation tests to occupational agents in the laboratory.

Assessment of allergenicity of chemical agents

Recently there have been attempts to develop an animal model that allows the assessment of occupational agents with the potential to induce airway hypersensitivity after exposure through inhalation. Initially a chemical is structurally analysed and its potential to bind to carrier proteins is assessed in vitro. This is followed by attempts at systemic sensitisation by injection in a guinea pig model and finally the effects of exposure by inhalation are assessed. It has been suggested that this tiered approach may allow chemicals to be graded according to their ability to induce sensitisation. This system does allow non-sedated and non-restrained animals to be used during periods of exposure by inhalation. Airway responses in sensitised animals are indirectly measured by assessment of changes in the pattern of breathing, which includes respiratory rate, tidal volume, and ratio of expiratory phase to inspiratory phase of respiration. Airway responsiveness to inhaled histamine may be similarly assessed. Immunological responses and histology must also be examined. Although such a model system is of considerable interest, there are some potential disadvantages. Whole body exposure allows for deposition of chemical agents onto the fur of the animal, in particular dusts, which may then be inhaled or ingested and induce sensitisation or airway responses in sensitised animals. Thus, the level and route of exposure may differ considerably from that intended. It may also be difficult with this model to distinguish a direct irritant or toxic effect of a chemical on the airways from that due to airway hypersensitivity alone. In humans, occupational asthma as a result of exposure to chemicals such as the isocyanates may occur without immunological evidence of sensitisation. Similarly in animal models, sensitisation and airway hypersensitivity to certain chemicals may occur in certain species in the absence of an obvious immunological response and in some species, in particular the guinea pig, the immunological basis for airway hypersensitivity responses is not fully understood. The absence of an obvious immunological response in animals exposed to a chemical need not imply that a particular chemical is incapable of inducing sensitisation of the airways. The development of such animal models, however, should allow further assessment of the exposure-dose relation that is important to the development of chemical sensitisation and to the subsequent development of airway hypersensitivity responses.

Conclusion

Although, all forms of occupational asthma may not derive from a uniform pathophysiology, it is now possible through the development of differing in vivo models to explore the various factors important in sensitisation and the subsequent development of immunologically mediated airway responses to low molecular weight chemicals.

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