THE EFFECT OF (1→3)-β-D-GLUCAN FROM BAKERS YEAST ON BRONCHIAL RESPONSIVENESS AND INDUCTION OF INFLAMMATION IN THE GUINEA PIG AIRWAY

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Abstract

(1→3)-β-D-glucans are a diverse group of naturally occurring glucose polymers. They have been demonstrated to have a range of stimulatory effects on various cell types including macrophages[1,2] and at an experimental level appear to have a role in respiratory changes induced by the inhalation of organic dusts[3].

Studies carried out by our group into the effects of inhalation of (1→3)-β-D-glucan (isolated from baker's yeast - glucan (BY)) demonstrate changes in airway responsiveness in the guinea pig airway. *In vitro*, there is a decrease in sensitivity to a variety of constrictors after exposure to glucan (BY)[4]. Changes in the histamine release characteristics of mast cells also occur after exposure to BY glucan[5].

In vivo changes in responsiveness occur in the guinea pig at certain time points post - glucan exposure. A significant eosinophilia has also been shown at several glucan dose levels. These findings will be discussed in relation to the role that glucans may play in the induction of environmental respiratory disease.

Introduction

(1→3)-β-D-glucans are a group of natural products found in the cell walls of various plants, fungi, moulds and some bacteria. They are a diverse family of compounds that differ greatly in their physical structure despite a common chemical element - they are composed of the monosaccharide - glucose. The glucose present in glucans is polymeric in nature and all glucans share a characteristic (1→3)-β-D backbone, branching of the polymer can occur generating a (1→6)-β side chain. Ultrastructurally, (1→3)-β-D-glucans occur in various forms, varying from random coils to single helices and stable triple helices depending on their degree of branching and the nature of the chemical interaction between the polymeric molecules.

The physical characterisation of (1→3)-β-D-glucans is important as it appears that such properties may influence their pharmacological activity. Thus, in studies of the effect of various glucans on markers of respiratory inflammation, it has been found that release of cytokines[1] , influx of inflammatory cells[7,8] and production of reactive oxygen species[2] within the airways are dependent on the molecular weight, degree of branching, ultrastructure and solubility of the (1→3)-β-D-glucans.

Respiratory diseases, such as asthma and byssinosis are characterised by airway hyperresponsiveness. This hyperresponsiveness can be characterised by two fundamental features both of which are of importance to the afflicted patient; airway inflammation (characterised by airway eosinophilia in the asthmatic) and also smooth muscle hyperreactivity which can be demonstrated experimentally and in the clinic. The aim of the present study was to establish whether (1→3)-β-D-glucans have any influence on the guinea pig airway *in vivo*. Can the phenomenon of airway hyperresponsiveness be induced using glucan in a model that is capable of demonstrating airway hyperresponsivess after exposure to another important agent derived from organic dust, lipopolysaccharide (LPS).

Methods

Drugs Used

The drugs used for the lavage (inflammation) study and the plethysmography (*in vivo)* were made up in non - pyrogenic, sterile saline (Baxter, Thetford, UK). The lavage vehicle used was phosphate buffered saline.

(1→3)-β-D-glucan (isolated from baker's yeast - glucan (BY)) and methacholine hydrochloride were obtained from Sigma Chemicals Ltd.

Animals and Husbandry

Male Dunkin Hartley guinea pigs of weight range of 350 - 550g were employed throughout. Prior to the experiments the animals were housed in groups of up to six animals in North Kent Plastic aluminium A1 guinea pig holding units (76 x 71 x23 cm) with stainless steel grid floors. The trays beneath the cages were lined with newspaper which was changed three times per week. Tap water, oranges and pelleted food (FDP; Special Diet Services, Bicester, Oxford) were available *ad libitum*. Temperature and humidity were maintained at 21^0c and 50% respectively. A light -dark cycle of 12h was maintained throughout.

Experimental Procedure

Male guinea pigs were placed in an exposure chamber for exposure to either nebulised I) 0.9% sterile saline solution or ii) (1→3)-β-D-glucan (at a concentration of 50 or 100mcg/ml in sterile saline) for one hour.

Methods (*In vivo*)

All animals used in the *in vivo* procedure were trained for use in the plethysmograph according to the method of Griffiths - Johnson *et al*[5]. Firstly, a baseline sGAW value

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was obtained for the response to the bronchoconstrictor methacholine (exposure to a 10mcg/ml solution occurred for 1 min using a Wright nebuliser operating at 20 p.s.i.) sGaw was measured 5min prior to exposure to the bronchoconstrictor and then also measured at 2,5,10 & 15min post exposure. The post - exposure sGaw divided by the baseline sGAW gave a percentage value for the initial reactivity (IR). Twenty four hours later, the guinea pigs were exposed to either saline or glucan (BY). At the appropriate time points the bronchoconstrictor challenge was repeated and a second reactivity value was calculated (NeR) and the change in reactivity value was therefore calculated as IR - NeR. The dose of methacholine given was judged to induce an IR of 30-40% so that decreases and increases in reactivity were detectable. Groups of six to eight animals were used for each exposure.

Methods (Lavage)

Animals were exposed to the test agents as described above. Each was given a lethal i.p. injection of pentobarbitone sodium (100mg/ ml). The thoracic area was cut, revealing the trachea which was incised and a Portex 6FG yellow Luer cannula inserted. The cannula was tied into place using surgical cotton and a 10ml syringe was attached to the free end. 5 x 10ml aliquots of phosphate buffered saline were introduced and removed in a gentle fashion to and from the lungs. The resulting suspension was used to perform differential and total cell counts providing that a greater than 80% of the lavage fluid was returned. This gave an indication of whether there was leakage of fluid from the lung and an assessment of the degree of physical damage suffered by the lung tissue due to experimental procedures.

Total Cell Count

A coverslip was placed on an improved Neubauer Haemocytometer. To obtain a total cell count a small amount of the suspension was drawn into a capillary tube an a drop placed at the edge of the coverslip. Capillary action drew the cell suspension into a 'monolayer' under the coverslip. The cells were allowed to settle for 1 min. For each cell suspension four counts were performed and the mean was calculated.

Differential Cell Counts

0.1ml of the cell suspension was taken and transferred to chambers prepared for use in a Shandon Cytospin III. The cells were spun at 100rpm for 6 min at the low acceleration setting. On removal they were dried in air by a fan. They were than fixed by placing in 95% ethanol for 5 mins. The slides were than redried and placed in Giemsa stain for 5 min. Following this they were washed with distilled water and placed in May - Grünwald stain for 2 min. The rinsing procedure was then repeated and left to dry. Differential cell counts of monocytes (mainly macrophages), eosinophils and neutrophils were performed counting at least three hundred cells per slide. All cells in the field of vision were counted and the slide was adjusted so that the next field of vision immediately to the right was obtained. This ensured the sample of cells counted was representative and reduces possible errors that may occur due to an uneven distribution of cells by the cytospin caused by the different masses of the cells.

Results

Figure 1 clearly illustrates the responses to inhalation of saline, glucan 50mcg/ml and glucan 100mcg/ml *in vivo*. Saline has been demonstrated to have no significant effect on airway responsiveness *in vivo* over the time period of 72h. Glucan 50mcg/ml caused a significant ($p<005$) increase in airway responsiveness at 1hr post exposure which was sustained until 4hr where a recovery to baseline values occurred leading to a complete return to normal responsiveness at 72hr. Glucan 100mcg/ml caused a similar, if more intense, hyperresponsiveness. This change in response was significant at both 1 and 4hrs post-exposure. After this initial period of increased response a complete recovery was made to baseline sGAW values at 72hrs.

Glucan at both concentrations used caused significant ($p<0.05$) increase in total cell count (figure 2), macrophage (figure 3) and eosinophil numbers (figure 4) recovered from broncho - alveolar lavage fluid. No significant change in total neutrophil number was observed above the value obtained for saline (figure 5).

Discussion

The results illustrated above clearly demonstrate the glucan (BY) is capable of inducing significant changes in airway responsiveness. The results highlight the fact that glucan (BY) can acutely affect the responsiveness of airway smooth muscle in such a way as to make the airways more responsive to given bronchoconstrictors such as methacholine. Glucans of this type therefore, either directly or indirectly, have the capacity to modulate the 'normal' functioning of intact airways.

Glucan (BY) has also been shown to be a significant agent involved in the recruitment of inflammatory cell types to the airway[8]. An increase in total, macrophage and eosinophil cell number recovered in broncho - alveolar lavage fluid was noted after acute glucan exposure. The development appears to precede the increases in macrophage and eosinophil cell numbers present in the airway, it is therefore unlikely that increased numbers of these cells are responsible for the induction of smooth muscle hyperresponsiveness. However, this study does not include an investigation into the degree of activation of these inflammatory cell types. The possibility therefore exists that as the cell numbers increase that their increased activation may be involved in smooth muscle responsiveness changes.

Glucan shares many similarities with another agent found in organic dusts: lipopolysaccharide (LPS). Both glucan (BY) and LPS cause a significant change in airway response 1hr post acute inhalation challenge[9]. LPS, however, proceeds to induce a phase of hyperresponsivess at 24 and 48hr post - challenge which coincides with the development of a significant neutrophilia[10]. Glucan (BY) by contrast is incapable of inducing the phase of hyporesponse and appears to induce a contrasting eosinophilia of the airway post challenge. An interesting question therefore arises as to what are the possible implications for the respiratory health of workers exposed to both agents?

Further work must be carried out using chronic as well as acute exposure to glucan (BY) to elucidate its' full potential as an agent capable of initiating or exacerbating organic dust induced diseases. Further studies in the fields of electron microscopy, co - administration challenges with other toxic agents such as tannins and LPS and the development of the basic knowledge of carbohydrate signalling in the immune and inflammatory responses will make clearer the role glucans may play in work place induced respiratory disease.

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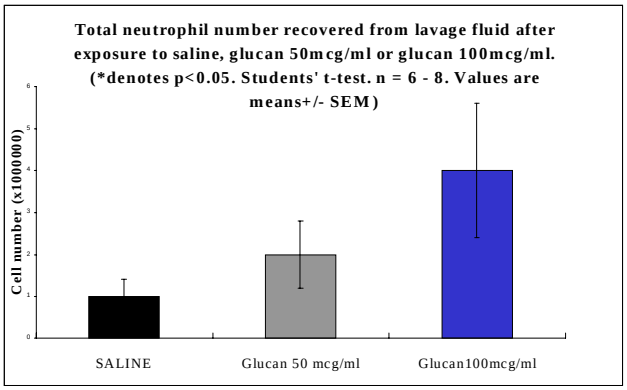

Figure 5. Chart 1