

Absence of IgG antibodies to TDI-HSA in a radioimmunological study

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Summary

Sera of ninety-one workers with respiratory symptoms related to occupational exposure to toluene diisocyanate (TDI) were tested by a solid phase radioimmunoassay using polyvinyl plates, in an attempt to demonstrate specific IgG antibodies to a conjugate of TDI with human serum albumin (TDI-HSA). Different conjugates were prepared. In our radioimmunoassay a TDI-HSA 1600 (1600 μ g TDI/ mg HSA), obtained at alkaline pH with borate buffer, was used; this conjugate was immunologically reactive and did not contain protein macro-aggregates.

No difference in percent of binding of radiolabelled anti-human IgG to TDI-HSA coated on plastic plates was found between exposed workers (asthmatic and non-asthmatic) and fifteen normal subjects.

This may suggest that either TDI-induced bronchial asthma is not mediated by IgG antibodies, or TDI-HSA conjugate is unsuitable to be detected by anti-isocyanate IgG.

Introduction

Isocyanate vapours have been known for many years to produce irritation of the skin, eyes and respiratory tract (Fuchs & Valade, 1951); it is also well known that very low concentrations of isocyanate (Pepys *et al.*, 1972; Butcher *et al.*, 1976) are able to induce bronchial asthma by an unknown pathogenetic mechanism. Studies aiming to demonstrate an immunological mechanism have led to discordant results. Specific IgG and IgE antibodies have been inconstantly detected in sera of exposed workers using various techniques, e.g. RAST and provided positive (Karol *et al.*, 1979; Zeiss *et al.*, 1980; Butcher *et al.*, 1980) and negative (Butcher *et al.*, 1977; Danks *et al.*, 1981) results in TDI asthmatic patients. The difficulties in the preparation of a complete antigen

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(isocyanate carrier) could, at least in part, explain the disagreement of the various reports.

This paper reports the results of a radioimmunological assay in an attempt to demonstrate anti-TDI-HSA antibodies in sera of TDI-exposed workers.

Materials and methods

Patients

Ninety-one workers with respiratory symptoms related to the occupational exposure to TDI were examined. Of these, thirty-eight were affected by TDI bronchial asthma, thirty-one by colds and twenty-two complained of upper airway symptoms. Control sera were from fifteen TDI non-exposed subjects. Sera of these patients were stored at -20°C until testing.

TDI conjugation to human serum albumin (TDI-HSA)

Scheel's technique (Scheel, Killens & Josephson, 1974), partly modified, was followed. Two millilitres of 2.4 TDI (Aldrich, Belgium) in dioxane were slowly added to the same volume of a constantly stirred, ice cold 10 mg/ml HSA solution (Calbiochemical, U.S.A.). The conjugate was then extensively dialyzed against PBS 0.01 M pH 7.4. Conjugation was confirmed by u.v. spectrophotometry. The TDI/HSA molar ratio of the conjugate was calculated from absorbance attributed to TDI at 255 nm and extinction coefficient of TDI.

Various conjugates were obtained using different concentrations of TDI in dioxane (from 200 to 1600 $\mu\text{g/ml}$) and different buffers at acid and alkaline pH. Immunological reactivity of HSA in the conjugate was evaluated by immunoelectrophoresis against an anti-total human serum from rabbit. The presence of macro-aggregates of TDI-HSA in the different conjugates was studied by chromatography on Sepharose 4B.

Solid phase RIA

The solid phase was a polyvinyl plate with ninety-six cups (Microtitre, S-MRC, Limbro Sc.) treated by the conjugate. After incubation at 20°C , it was removed by aspiration. The cups were also saturated with 1% BSA in PBS. After washing with PBS, 1:5 diluted sera were added and allowed to incubate for 1 hr at room temperature; sera were then removed and the cups washed. One hundred microlitres of 1% BSA in PBS containing about 50 000 ct/min of rabbit ^{125}I anti-human IgG antibodies, purified by immunoabsorbent and labelled by the lactoperoxidase technique (Marchalonis, 1969) were added in each cup and allowed to incubate overnight. Radioactivity bounded was expressed as percent of total.

Human serum albumin at the same concentration of TDI-HSA was used as antigen in the same experiments.

Results

Quality of the TDI-HSA conjugate

Figure 1 shows the absorbance at 255 nm of the conjugates obtained at different pH; a higher value was found in the acid procedure.

Chromatograms of the different conjugates were compared to the chromatograms of HSA 5 mg/ml and 0.2% dextran blue. No aggregates were present in the conjugates

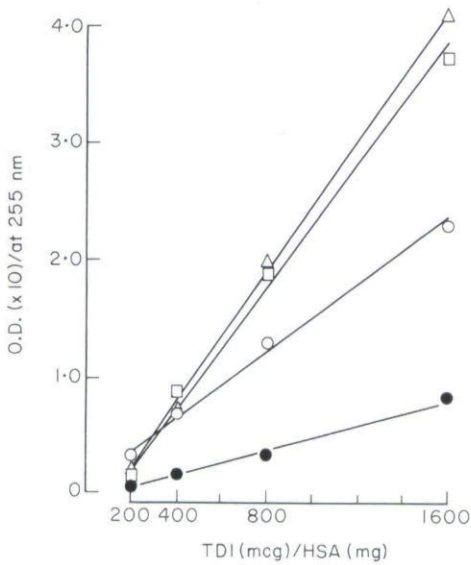


Fig. 1. The u.v. absorbance at 255 nm of the conjugates obtained in different conditions of pH and TDI/HSA molar ratio is reported. Δ , TDH-HSA, pH 4.5 acetate buffer; $\square$, TDI-HSA, pH 4.5 HCl; $\circ$, TDI-HSA pH 9.5 NaOH; $\bullet$, TDI-HSA, pH 9.5 NaOH borax buffer.

obtained at alkaline pH, but macro-aggregates were found in those obtained at acid pH and may explain the higher absorbance of the last ones.

Immunological reactivity of HSA in the conjugates was preserved, as shown by immunoelectrophoresis against a rabbit anti-human serum.

IgG anti-TDI-HSA

TDI-HSA 1600 obtained at alkaline pH with a 34.2 TDI/HSA molar ratio, was used for coating the plastic cups. The minimal concentration of the conjugate for coating was 0.15 mg/ml, this was used throughout the investigation.

Figure 2 shows the percent of binding on TDI-HSA of sera from non-exposed, asthmatic and non-asthmatic subjects. No difference was evident between them. No difference was found between sera of twenty asthmatic and twenty non-asthmatic

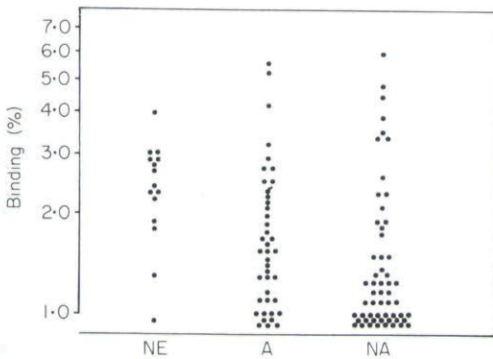


Fig. 2. The percent of binding in sera of non-exposed (NE), asthmatic (A) and non-asthmatic (NA) subjects.

subjects when the percent of binding was calculated as difference between the binding on TDI-HSA and HSA alone.

Discussion

TDI-specific IgG antibodies have been detected in moderately high percent of sera of TDI-exposed workers by various techniques, e.g. gel diffusion and passive haemagglutination (Taylor, 1970; Saia, Chierchia & Brugnone, 1972; Nava *et al.*, 1975; Butcher *et al.*, 1980; Nava & Briatico Vangosa, 1980). By a radioimmunoassay technique Zeiss *et al.* (1980) found anti-MDI-HSA IgG antibodies in two workers with MDI-induced bronchial asthma.

In all studies HSA was used as carrier, following Scheel's technique, but changes in immunological properties of HSA and the presence of macro-aggregates after TDI conjugation have not been described adequately. TDI-HSA macro-aggregates in the conjugates may induce a false evaluation of TDI-HSA molar ratio by means of u.v. spectrophotometry and cause non-specific precipitation in gel diffusion. We demonstrated that the alkaline conjugation in borate buffer does not induce the appearance of TDI-HSA macro-aggregates.

Our radioimmunoassay does not show the presence of TDI-specific IgG antibodies in sera of asthmatic and non-asthmatic subjects exposed to isocyanates. This fact may have various explanations: (1) our method is not sufficiently sensitive to detect a low antibody titre; nevertheless when using a different antigen the same method has been shown to discriminate normal sera from slightly positive sera; (2) the pathogenetic process of TDI-induced bronchial asthma is not mediated by IgG antibodies; (3) our TDI-HSA conjugate is unsuitable to be detected by anti-isocyanate IgG. In effect the discordant results obtained by other authors using the same or different conjugates may reflect the variability in their antigenic properties. These observations underline the role of both the carrier and the hapten in recognition of the complete antigen by specific anti-isocyanate IgG.

In conclusion we cannot establish if the failure detect antibodies is due to the absence of antibodies in TDI asthmatic patients or the lack of suitable antigen determinants in our conjugate.

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