Immune Complex Anaphylaxis Induced by Dextran and Its Elimination by Hapten Inhibition

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Abstract

Purpose: To elucidate the mechanism of the rare anaphylactic reactions elicited by i.v. infusion of Macrodex® and Rheomacrodex® and to find measures for their elimination.

Methods: Hapten inhibition in vitro and in dextran anaphylaxis in guinea pigs and dogs.

Human serology: Complement profiles; IgE assays: RAST, PCA in monkeys, RCLAAR*; estimation of dextran reactive antibodies (DRA) by gel diffusion, passive hemagglutination, RCLAAR for IgG and subclasses, IgA, IgM, IgD; ELISA for IgG. Histopathology of human lung specimens. Clinical trials in five countries to assess efficacy of hapten inhibition.

Summary of results: Dextran anaphylaxis (incidence 0.05%) occurs upon *the first* infusion of a few ml of clinical dextran. Severity of anaphylaxis positively correlates with IgG-DRA levels (especially IgG_2) reaching conc of 1 mg/ml. High titers of specific IgA and IgM were also found *but no IgE* or IgD. C1q levels were low. Lung histopathology of fatal cases disclosed obstruction of vessels with globuli of fibrinlike material, aggregated platelets and leukocytes. The naturally occurring DRA which cause anaphylaxis originate from immunization with ingested wild type dextran or cross-reactive bacterial polysaccharides. Since dextran anaphylaxis in animals could be prevented or reduced by hapten inhibition, and a dextran fraction of M_w 1000 (Promit®) proved to be non-eliciting and safe, clinical trials were started. 130000 patients were treated with injection of 1.5 or 3 g of Promit® 2 min prior to clinical dextran infusion. The 3 g dose reduced the incidence of anaphylaxis about 20 times. This was confirmed by a two years post-marketing surveillance period in Sweden.

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* Red cell linked antigen antibody reaction.

J. Ring et al. (eds.), *New Trends in Allergy II*

Conclusions: Dextran anaphylaxis is an immediate type, IgG-mediated reaction comparable to anaphylaxis in patients with deficiency of IgA or factor VIII. Hapten inhibition could be successfully applied to eliminate life-threatening dextran reactions by injecting monovalent hapten-dextran (Promit®) prior to infusion of Macrodex®/Rheomacrodes®.

Introduction

In recent years the worldwide use of pharmaceuticals with established medical value has imposed increased demands on their safety. Among plasma substitutes the efficacy of dextran is well documented. Its additional blood flow improving and thromboprophylactic effects are widely used. Although severe adverse reactions to clinical dextran preparations are rare [1], they occur with an estimated incidence of 1:2000 patients [2]. The elimination of such reactions was therefore made the aim of studies begun in Uppsala in 1968. Since then, many workers have joined the research program and formed collaborating study groups in Munich, Uppsala, and Vienna. Many publications have reflected the progress of the project [3–24; for reviews see 14, 21, 22, 24, 43]. The stepwise elucidation of the mechanism of dextran anaphylaxis in man, the study of animal models of dextran anaphylaxis, and its successful prevention by hapten inhibition, as well as the production of monovalent hapten-dextran on a large scale [18] enabled us to begin clinical trials of hapten prophylaxis in humans in 1978. The trials grew into large multicenter multinational studies, which ended in 1982 and were published in a series of articles [24, 33–40]. Results show that the incidence of severe dextran-induced anaphylactic reactions (DIARs) is greatly reduced by preinjection of hapten-dextran in a dose-dependent manner, conferring greater safety to dextran infusion therapy. In this chapter I shall outline the course of the research program and the present data from the combined clinical trials and subsequent postmarketing surveillance in Sweden (1983–1984). The role of IgG-mediated anaphylactic reactions, as opposed to IgE-mediated ones, will also be discussed.

Historical Background

Dextran of $\bar{M}_w$ 70000 was introduced into medicine as plasma substitute in 1947 by Ingelman and Grönwall [6]. It was prepared from a branched type of native dextran and quite frequently caused mild allergic reactions. Change to a more linear dextran reduced the incidence of allergic reactions [25]. This dextran produced by the *Leuconostoc mesenteroides* NRRL B 512 strain is still used today.

Gelin and co-workers showed that dextran of $\bar{M}_w$ 40000 increased the suspension stability of blood and improved blood flow in the microcirculation [26]. This led to the introduction of Rheomacrodex as a blood flow-improving agent [27]. In the 1960s an additional effect of dextran 70 was found, i.e., that it reduced the incidence of postoperative pulmonary embolism [28]. This thromboprophylactic effect is now the most frequent medical indication for dextran 70. The increased worldwide use of clinical dextran led to a considerable number of reported adverse reactions in spite of the low incidence of such reactions.

Immunology of Dextran

The immunogenicity of B 512 dextran in man is molecular weight dependent [29]. Whereas native and very high molecular weight dextrans are immunogenic in humans, dextran 70 and 40 are nonimmunogenic [21]. From the immunological point of view, a dextran infusion with 30–100 g represents an "overwhelming" dose. In animals corresponding doses lead to immunological unresponsiveness. Like other polysaccharides, dextran induces a thymus-independent IgM response in mice [21]. However, dextran can be converted to a thymus-dependent antigen by covalent coupling to protein [5]. Such conjugates elicit a strong IgG antidextran response upon immunization of carp, mice, guinea pigs, rabbits, sheep, and horses. We have utilized this fact to raise antidextrans in rabbits and dogs for use in our anaphylaxis models and for analytical purposes [5, 8]. Most people have natural dextran-reactive antibodies (DRAs) in their sera. They may have been induced by native, high molecular weight dextran ingested as food contaminant, produced by bacteria of the gastrointestinal tract, or produced by other cross-reactive microbial polysaccharides [30; for review see 21].

Table 1. Scale of severity of clinical symptoms of adverse reactions to colloidal infusion solutions (Rind and Meßmer [14], Laubenthal [36])

Grade of severity	Clinical symptoms
I	Skin manifestations: flush, erythema, urticaria
II	Measurable, but not life-threatening hemodynamic reaction (blood pressure fall, 20–60 mmHg). Dyspnea, nausea, vomiting
III	Shock (blood pressure fall exceeding 60 mmHg). Life-threatening bronchospasm
IV	Cardiac and/or respiratory arrest

Serology in Humans

Antibodies

Analysis of sera of dextran reactors and nonreactors, accumulated over many years, with various standardized procedures for measuring DRAs, gave the following results. Circulating DRAs occur in low titers in the majority of human populations; high titers are only found in a small percentage of individuals. Geographical variations in titer distribution do occur [23]. All patients with severe DIAR of grades III and IV (see Table 1 for classification) have high or very high titers of circulating DRA before the reaction. No IgE class DRAs could be found in 100 dextran reactors with different methods [7]. When Ig classes and subgroups were studied by RCLAAR and ELISA in reactors and nonreactors to dextran, high titers of IgG, especially of IgG_2, usually accompanied by IgA and IgM, were found in reactors, whereas nonreactors had small amounts of mostly IgM class DRAs [11, 19]. An excellent correlation was found between the grade of severity of DIAR and the titer of IgG class DRAs, implying that large amounts of immune complexes are formed in the circulation of patients with the most severe reactions [19].

Complement

Complement profiles were established in dextran reactors [7]. The most important finding was a significant decrease in the levels of Cl_q in severe DIAR. Concentrations of the other complement proteins and of the anaphylatoxin inactivator were normal. These results show that the classical pathway is activated in dextran reactors by immune complexes. The findings are in accord with the occurrence of high titers of IgG class DRAs in patients with severe DIAR.

Studies of the Chemical and Pharmaceutical Manufacturing Procedure

Dextran is a polysaccharide produced by the action of *Leuconostoc mesenteroides* NRRL B 512 on sucrose in the presence of nutrients. Thus, a careful purification process is necessary to manufacture clinical dextran preparations of defined molecular weight and molecular weight distribution. Since macromolecular contaminants may sensitize patients, we tested the immunogenicity of dextran from the early stages of the manufacturing process. No evidence for immunogenic impurities could be demonstrated [5]. Later, it was found that a soluble macromolecular component of *Leuconostoc* bacteria reacted with anti-yeast mannan antibodies. As such a *Leuconostoc*-derived component could be a potentially sensitizing agent in clinical dextran, an im-

munochemical test for its detection and quantitation was developed [31]. By improved purification, its concentration in clinical dextran was reduced to 10 ppm or less. Comparison of the incidence of adverse reactions to clinical dextran before and after introduction of the *Leuconostoc* RSRI purity test provided no evidence for a causal role of this contaminant in eliciting DIAR, but suggested an elicitor role of the dextran molecule itself.

Hapten Inhibition In Vitro

Haptens cannot induce antibody formation but do bind to antibodies of corresponding specificity. They may be poly-/or monovalent with regard to the number of antigenic determinants. Whereas a polyvalent hapten forms immune complexes with antibodies, a monovalent hapten binds to individual combining sites of antibodies only, and cannot join together antibodies by bridging. In the B 512 dextran-antidextran system, monovalent isomalto-oligosaccharides (IOS) inhibit the precipitation of antidextran by large dextran molecules [32]. Their inhibitory power increases strongly from isomaltose to isomaltopentaose with little further increase for isomalto-hexaose and isomalto-heptaose. These results were confirmed by indirect single radial immunodiffusion [5]. A dextran fragment of 6 glucose units ($\bar{M}_w$ 990) was judged suitable as a monovalent hapten for in vivo experiments. Thus, dextran 1 with $\bar{M}_w$ 1000 was prepared on a large scale by prolonged acid hydrolysis of B 512 dextran with subsequent fractionation and purification [18]. It is a mixture of IOS with 2−13 glucose units. Its molecular size distribution is controlled by gel chromatography. Dextran 1 is used clinically as a sterile 15% solution delivered in 20-ml vials.

Animal Experiments on Anaphylactic Shock

Hapten Inhibition of Cytotropic Passive Dextran Anaphylaxis in Guinea Pigs

The most important findings show that 100% protection from anaphylactic death can be achieved by admixture of low molecular hapten-dextran to the challenging high molecular dextran or by injection of hapten-dextran prior to challenge [3]. The protective effect is dose dependent and a significant reduction in mortality is observed when the hapten is present in a molar excess of 3−7, corresponding to an admixture of 10%−50% w/w to the challenging dextran [3, 4]. For the hapten, a $\bar{M}_w$ range of 1000 was found to be optimal in terms of both protective effect and safety requirements [5]. No elicitor action is present upon challenge with such a hapten, even at maximal degrees of sensiti-

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