Allergology International (2003) **52**: 165–170

Hypothesis

A hypothesis for the mechanism of immediate hypersensitivity to mannitol

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Abstract

Immediate hypersensitivity reactions to mannitol present naturally in pomegranate (*Punica granatum*) and cultivated mushroom (*Agaricus bisporus*) have appeared in the medical literature recently. Mannitol, being inert, cannot react with proteins to form a hapten–carrier conjugate and elicit an immune response. Therefore, it is important to understand the mechanism of immediate hypersensitivity to this sugar alcohol. A likely mechanism was conceptualized to explain how an individual can become sensitized to mannitol and how free mannitol can elicit an anaphylactic reaction in the sensitized individual. The proposed mechanism for sensitization involves the reaction of D-mannose with exposed amino groups of proteins *in vivo* to form Schiff base intermediates bearing a D-mannitoyl moiety, which closely resembles D-mannitol. This intermediate appears to be responsible for eliciting the formation of mannitol-specific IgE in susceptible individuals. Once an individual is sensitized with the formation of mannitol-specific IgE, mannitol can cause anaphylactic reactions by acting either as a univalent anaphylactogen or a bivalent hapten. The Schiff base intermediate bearing the mannitoyl moiety appears to act as a true sensitizer, whereas D-mannose appears to act as prosensitizer and D-mannitol acts as a non-sensitizing elicitor. This hypothesis can also explain the mechanism of sensitization and IgE-mediated hypersensitivity to any sugar alcohol.

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Received 2 December 2002. Accepted for publication 19 March 2003.

Key words: anaphylactogen, hapten, hypersensitivity, mannitol, nonsensitizing elicitor, prosensitizer, sensitizer.

Introduction

Mannitol, a non-toxic and non-metabolizable osmotically active compound, is administered as a therapeutic agent in many clinical situations, such as drug intoxication, oliguric renal failure, glaucoma and increased intracranial pressure. Mannitol is widely used as a food additive in processed foods and as an excipient in pharmaceutical preparations. Hypersensitivity reactions to 10 or 20% (w/v) mannitol intravenous infusion have been reported in the medical literature.[1–7] These appear to be anaphylactoid reactions caused by direct action of mannitol at hyperosmolar (>100 mmol/L or 1.8% w/v) concentrations on mast cells/basophils.[3,8] In the seven cases described from North America and Europe (age range 16–65 years), symptoms varied from severe urticaria, wheezing and hypotension to loss of consciousness and anaphylactic shock.[1–7]

Mannitol occurs naturally in many plant foods. Recently, we identified mannitol, present in pomegranate (*Punica granatum*) and cultivated mushroom (*Agaricus bisporus*), as a low molecular weight (LMW) allergen responsible for anaphylaxis in a 32-year-old sensitized subject.[9,10] Symptoms of anaphylaxis included generalized urticaria, angioedema, difficulty in breathing and loss of consciousness. The identification of mannitol as the LMW allergen was based mainly on positive double-blind placebo-controlled food challenge (DBPCFC) using 1% pomegranate juice and positive skin prick test (SPT) with authentic mannitol, as well as mannitol isolated from pomegranate and mushroom. The mannitol content of fresh mushroom is 1.15% w/w; its content in pomegranate was quantified as 0.25% w/w.[9] Other common

sugars (including D-mannose) and sugar alcohols (including sorbitol) were all negative in the SPT,[10] indicating that the allergic subject was sensitized specifically to mannitol.

Sorbitol, xylitol, erythritol, maltitol and lactitol are other common sugar alcohols (also termed polyhydric alcohols or polyols) used as food additives. Erythritol is currently in use in Japan as a non-caloric sweetener.[11] Three cases of hypersensitivity reactions have been reported recently for erythritol present in foods and beverages[12,13] (one from Japan and two from North America) and a possible IgE-mediated anaphylactic reaction to haptenic erythritol has been suggested.[13]

Small molecules act as haptens in producing immediate (type I) hypersensitivity reactions. A non-immunogenic small molecule (molecular weight <1000 Da) must bind to carrier macromolecules, which are usually proteins (soluble or cell-bound proteins, including major histocompatibility complexes and associated peptides), before eliciting an immune response.[14,15]

HYPOTHESIS

Mechanism of sensitization to mannitol

Allergy to a small molecule depends on its reactivity with proteins to form an effective hapten–carrier complex to induce an immune reaction. Mannitol does not have a reactive group by which it can bind covalently to macromolecules such as proteins and act as a hapten; however, mannitol can bind non-covalently to a specific-binding protein, such as a lectin or a transporter. However, because mannitol is a very small molecule, even if it binds non-covalently to a specific hydrophilic pocket of a lectin (or mannitol transporter), this mode of interaction may render the mannitol epitope unavailable to elicit an immune response. Therefore, such non-covalent interactions appear unlikely for hapten sensitization.

In order to explain the mechanism of sensitization to mannitol, we saw the necessity to invoke a biotransformation product, which resembles mannitol (see Fig. 1) structurally to a large extent. It is well known that a small proportion of any aldohexose in solution exists in acyclic form (with a free aldehyde group) in equilibrium with the cyclic pyranose form (Fig. 1a). Our proposal is that the aldehyde group of the acyclic form of the reducing sugar D-mannose (Fig. 1b) can form a Schiff base by its reaction with exposed amino groups of proteins *in vivo* (Fig. 1). The Schiff base intermediate (Fig. 1c) bearing mannitoyl epitopes can elicit a specific immune response producing mannitol-specific IgE because the epitopes are available as antigenic determinants on the protein surface. In this context, D-mannose appears to be acting as a prosensitizer and the Schiff base intermediate appears to be the true sensitizer.

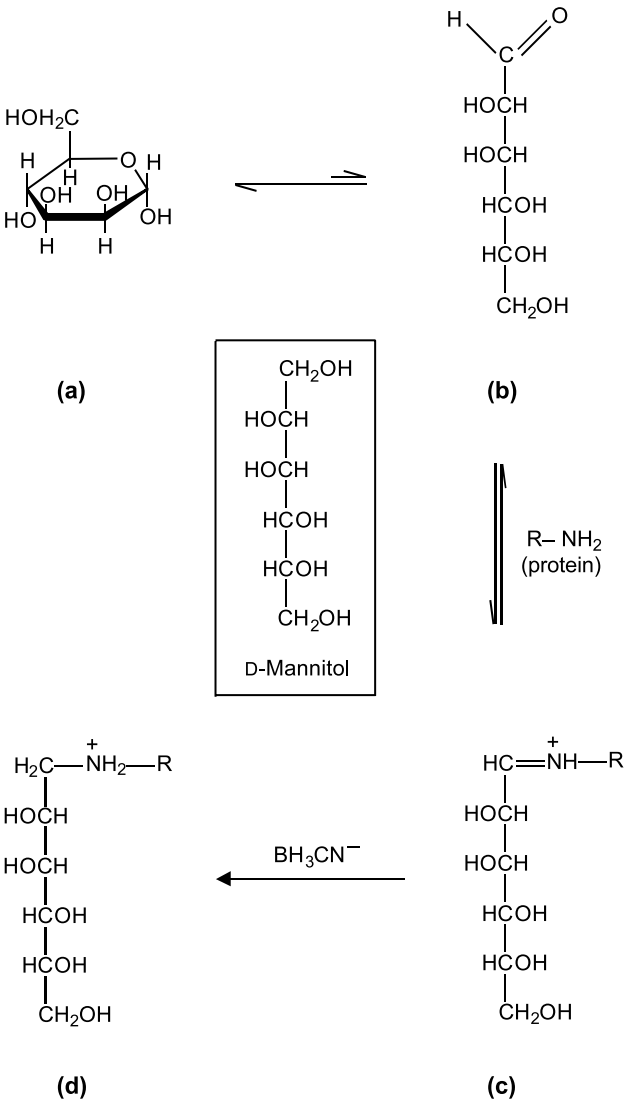

Fig. 1 Reaction scheme for non-enzymatic mannosylation of proteins in the formation of mannitoyl epitopes. The structure of D-mannitol is shown in the box (carbons C1 to C6 should be viewed from top to bottom). In solution, D-mannose (a) exists in equilibrium with minute amounts of its acyclic (straight chain) form (b), which can react with amino groups of proteins *in vivo* or *in vitro*, at or above neutral pH, to form unstable Schiff base intermediates (c). In the presence of sodium cyanoborohydride, the Schiff bases are reduced to stable mannitoyl derivatives (d) *in vitro*.

The proposed mechanism is based on earlier studies, which have established the reaction of D-glucose with a free N-terminal α-amino group or ε-amino groups of lysine residues of various proteins, such as serum albumin, hemoglobin, plasma lipoproteins, lens crystallins, collagen and myelin *in vivo*, to form Schiff bases.[16] At physiological pH, this reaction is initiated by the nucleophilic attack of amino groups on the carbonyl carbon of the acyclic form of sugar, leading to the formation of a Schiff base (also termed an aldimine intermediate). The reaction occurs extremely slowly, because only a very small proportion of the reacting hexose is in the acyclic form. The unstable Schiff base then undergoes Amadori rearrangement to produce the more stable ketoamine and hemiketal derivatives of proteins. The overall process, termed non-enzymic glycation, is an early stage of the Maillard reaction[16] observed in diabetes due to elevated levels of blood glucose. In the case of mannitol hypersensitivity, the sensitization may have occurred after consumption of mannose-rich foods (containing either free mannose or mannans/mannosans, which are polysaccharides of mannose), such as members of the custard apple, ebony and palm families, and processed foods/pharmaceuticals containing mannans/mannosans or to exogenous/endogenous aldimine intermediates of mannose in a situation analogous to the Maillard reaction of glucose with proteins.

Mechanism of anaphylactic reaction to free mannitol

Our observations indicate that mannitol hypersensitivity in the sensitized subject was mediated by mannitol-specific IgE and that pure mannitol can also elicit an anaphylactic reaction.[9,10] It is important to understand how an inert, simple molecule such as mannitol can elicit an immediate response because its size is similar to that of a single antigenic determinant. Two likely mechanisms to explain the anaphylactogenicity of mannitol are provided below.

Does mannitol act as a bivalent hapten?

The structure of D-mannitol is internally symmetrical and can be visualized as containing two identical 3-carbon polyol units (Fig. 2). Based on this unique structure, it can be speculated that mannitol may act as a bivalent hapten and can, by itself, potentially cross-link cell-bound specific IgE. This is a possibility, because a structure as small as a formaldehyde epitope (in a formaldehyde–protein adduct) has been shown to elicit specific IgE in humans.[17–20] In addition, acetaldehyde, which is a major metabolic product of ethanol, is known to form stable adducts with plasma proteins. It has been demonstrated that immunization of mice with protein–acetaldehyde adducts in aluminum hydroxide gel resulted

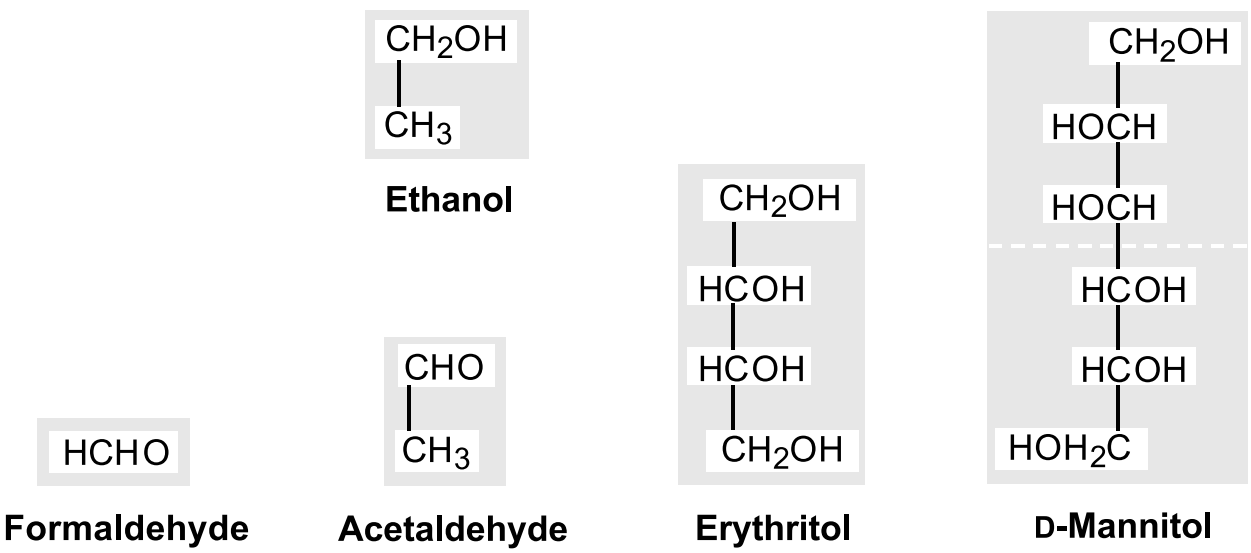

Fig. 2 Chemical structures of some small molecules to which immediate hypersensitivity reactions have been reported.

in the production of reaginic antibodies that recognize the adducts and trigger an allergic–anaphylactic reaction.[21] Furthermore, it has been shown that individuals with severe hypersensitivity reactions to ethanol have elevated levels of circulating anti-acetaldehyde–protein IgE antibodies.[22–24] In these studies, the acetaldehyde moiety has been shown to be acting as a hapten. It is also interesting to note that erythritol, a 4-carbon polyol, has recently been shown to cause immediate hypersensitivity in some individuals,[12,13] whereas there are no reports of hypersensitivity reactions in the literature to other commonly used sugar alcohols, such as sorbitol and xylitol.

Does mannitol act as a univalent anaphylactogen?

The cross-linking of IgE antibodies ligated on FcεRI on mast cell/basophil surfaces by bivalent or multivalent allergens has been strongly believed as the central dogma of immediate hypersensitivity reactions.[25] Nevertheless, there is ample evidence that a one-to-one interaction of antibody and hapten may suffice to elicit anaphylactic response by an IgE-mediated, cross-linking-independent mechanism in some exceptional cases.[26–30] Such haptens are usually referred to as univalent anaphylactogens or univalent elicitors. Common examples of univalent anaphylactogens are

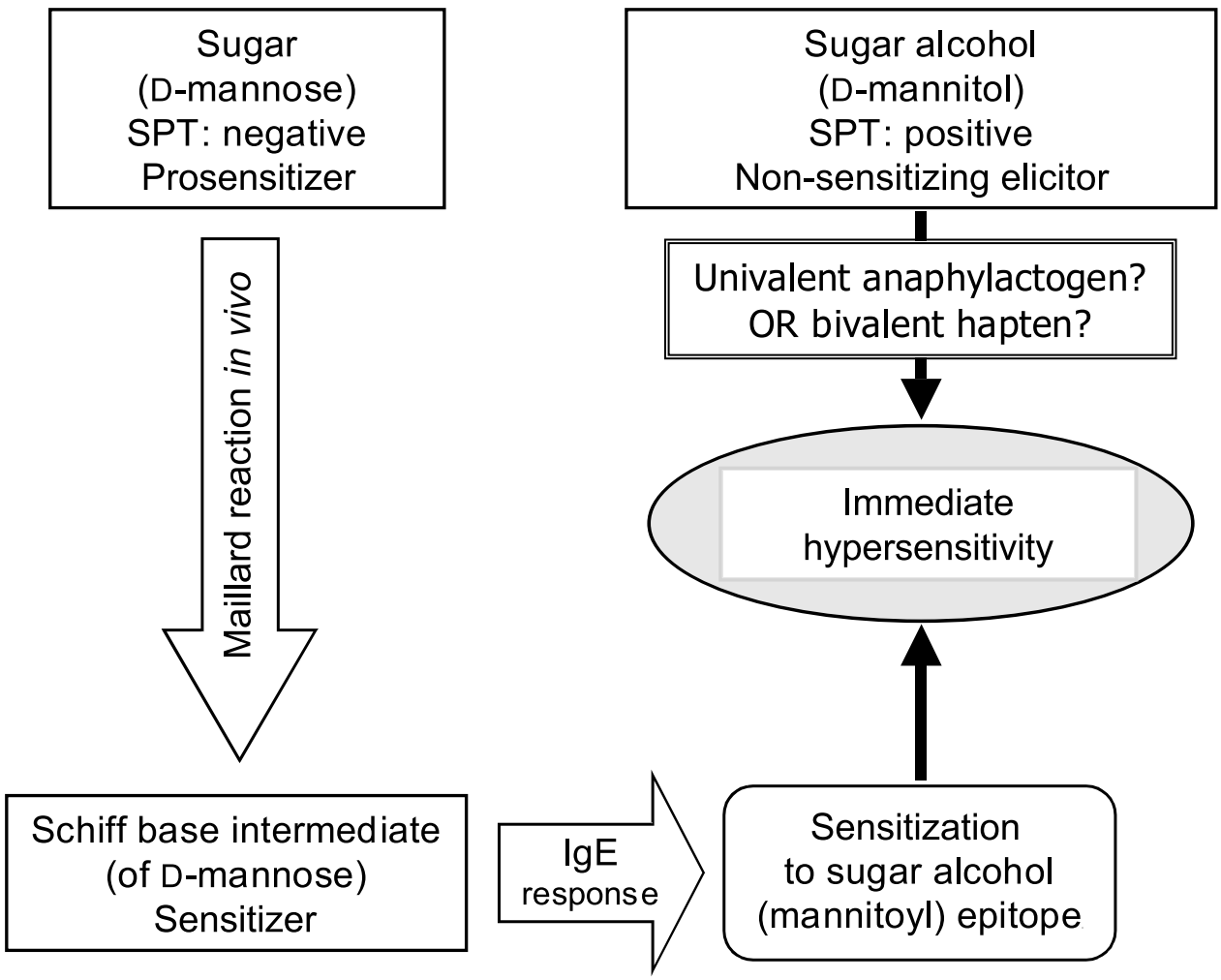

Fig. 3 Hypothesis to explain the basis for immediate hypersensitivity to mannitol. Skin prick test (SPT) results are taken from Hegde *et al*.[9,10] Positive SPT denotes the presence of mast cell-bound specific IgE.

the dinitrophenyl group (including 2-carboxy-4,6-dintrophenyl), *p*-azobenzenearsonate derivatives, penicillin and its derivatives. Once an individual is sensitized with the formation of mannitol-specific IgE antibodies by the mechanism proposed earlier in this paper, D-mannitol can act as a univalent elicitor, resulting in an anaphylactic reaction.

Conclusions

The hypothesis put forward here should explain, in principle, the mechanism of sensitization and of IgE-mediated hypersensitivity to any sugar alcohol. In such cases of hypersensitivity to a particular sugar alcohol (a non-sensitizing elicitor), the corresponding sugar acts as a prosensitizer in generating the Schiff base intermediate, which acts as a true sensitizer. The postulated mechanism of sensitization and the mode of action of mannitol in eliciting immediate hypersensitivity are summarized in Fig. 3. It appears that mannitol causes anaphylactic reactions in sensitized individuals by acting either as a univalent anaphylactogen or as a bivalent hapten. Based on the description of cases of mannitol hypersensitivity in the literature, it is evident that none had sensitivity to D-mannose, erythritol or any other related compounds. Hence, it is likely that mannitol-specific IgE antibodies may be very specific, without any cross-reactivity.

Acknowledgments

Supported by Council of Scientific and Industrial Research (CSIR), New Delhi, Institutional Project No. MLP-1407 on 'Allergens from Fruits' (YPV) and a Senior Research Fellowship (VLH).

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