

Serological Investigation of Latex Allergy in Argentina

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ABSTRACT

Atopy to latex has been reported in different high-risk groups of subjects in whom it is mainly caused by proteins from natural latex that are responsible for eliciting a variety of clinical symptoms, some of them systemic. Thus, identification of subjects sensitized to latex proteins is of great health importance, because it would become possible to advise them to avoid contact with latex-containing products. Protein extracts from ammoniated latex were prepared by incubation with PBS buffer either with or without detergents, followed by ultracentrifugation. Three immunochemical methods were developed (EAST, ELISA, and immunoblotting) to detect the presence of specific IgE antibodies against latex proteins in sera of patients from different groups at risk. The protein content of the latex extracts ranged from 5.3 to 8.8 mg/mL. The prevalence of specific IgE against latex proteins was 9/28 (32.1%) in children with multiple surgeries, 17/98 (17.3%) in health care workers, and 23/123 (18.6%) in outpatients assisted at the Allergy Department. None of the sera belonging to the healthy control group showed the presence of specific IgE. Therefore, this protein extract from latex could be used to detect specific IgE antibodies in serum by immunochemical methods. Serologic results for latex-specific IgE found are in accordance with those reported in the literature of other countries. (Allergy and Asthma Proc 20:99–106, 1999)

Allergy to latex proteins has been described in different high risk populations such as latex industry workers,¹ health care workers,^{2,3} patients with multiple surgeries (children with spina bifida,^{4,5} neurologic disorders, and congenital urologic abnormalities), and atopic patients,

mostly patients allergic to some fruits.^{6,7} The source of sensitization in Type I hypersensitivity appeared to be native proteins of the natural latex sap that may remain in latex products,⁸ and are responsible for the IgE-mediated reactions to a variety of latex products such as gloves, balloons, toys, catheters, condoms, pacifiers, dental dams, etc. Type IV hypersensitivity has been mainly ascribed to chemical additives in rubber products.⁸ The protein content of these products varies as a result of the different processes used in their manufacture; and so does their allergenicity.

Natural latex contains about 1.5–3% of proteins with a molecular weight (MW) ranging from 5 to greater than 100.^{8,9,10} It is well known that rubber products contain water-soluble and thermostable proteins that may cause Type I hypersensitivity. Multiple proteins have been identified as allergens, and some of them have been cloned and partially characterized. Structure, biologic activities, and immunochemical properties require further research. The prevalence of latex sensitivity has been reported since 1979 and it has become an increasingly occupational problem among glove-wearing health care workers. Moreover, latex allergy also occurs outside the medical community in the general population, where a prevalence rate of less than 1% was found.¹¹ Therefore, it is important to develop tests to identify individuals sensitized to latex proteins, as well as to determine the potential allergenicity of the latex products to which they are exposed. Many authors reported that sensitized patients may develop atopic signs after contact with latex-made products or inhalation of latex-airborne allergens. Reports show that the symptoms range from mild (contact urticaria, rhinoconjunctivitis, mucosal swelling) to systemic (generalized urticaria, asthma, and anaphylactic shock).^{12,13,14}

The presence of latex-specific IgE in allergic patients has been verified by in vivo and in vitro tests, mainly skin-prick test (SPT) and radioallergosorbent test (RAST). Actually, seven proteins have been cloned and given allergen designations by the International Union of Immunologic Societies. Several authors have identified a protein pattern to

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which patients with spina bifida are specially sensitive.^{4,5,15} Moreover, it has been reported that the protein associated with the surface of rubber particles, rubber elongation factor (REF) or *Hev b 1*, would be the major allergen in the group of the children with spina bifida,^{16,17} whereas *Hev b 2* behaves as a minor allergen for this group.¹⁸ Other authors were unable to demonstrate the allergenicity of the 14-kDa protein.^{19,20} Some reports show that the prohevein or *Hev b 6*, a 20-kDa protein, is the major allergen in adult allergic patients,^{20,21} and finally, a 27-kDa fraction *Hev b 3*, was found to be an important allergenic component for children who underwent multiple surgical procedures.^{4,5} Recently, *Hev b 7*, a 46-kDa protein, has been cloned and characterized as an allergen. *Hev b 5* was described to cross-react with antigens from kiwi and potato. *Hev b 3* and *Hev b 4* are less well characterized.²²

We have prepared a protein extract from natural rubber latex, with the appropriate allergens for serologic assays, to detect the presence of specific IgE in the sera of latex-allergic patients. Three immunoassays (ELISA, EAST, and immunoblotting), have been developed to analyze the sera of patients belonging to different high-risk groups (children with multiple surgeries, health care workers, and outpatients of the Allergy Department) in Argentina, where no information is available at present. It was not possible to assign a definite immunoblot pattern of reactivity for each group. The clinical relevance of this finding should be evaluated in further studies.

MATERIALS AND METHODS

Latex Source and Extract Preparation

Low ammoniated latex (0.22%) from Malaysian *Hevea brasiliensis* trees (Lee Latex LTD, clones CC 547 and SS 72) was kindly supplied by KOPELCO SA (Buenos Aires, Argentina).

Water soluble proteins from unprocessed latex were extracted by incubation with phosphate-buffered saline pH 7.4 (1:1) either without or with different types of detergents: 0.5% Tween 80, 1% SDS, 0.5% Triton X100, 0.5% NP40 or 1% deoxycholate.

Incubation was performed for 12 hours, and samples were then centrifuged at $100,000 \times g$ for 90 minutes at 4°C. The rubbery upper layer was discarded. The yellow aqueous layer (*C serum*) was dialyzed overnight using a 2-kDa cutoff membrane (SPECTRA/POR MWCO 2000) against Tris buffered saline, followed by filtration through a 0.22 μ m membrane filter. Extracts were stored at -20°C until used.

Total protein concentration was determined by the modified Lowry method with bovine serum albumin as the assay standard. Proteins were precipitated with trichloroacetic and phosphotungstic acids.²³

Electrophoresis and Immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins extracted from ammoniated latex was carried out in a 15% polyacrylamide gel with a discontinuous buffer system according to Laemmli.²⁴ The run was carried out at 100 V for the stacking gel and at 150 V for the running gel either with or without β mercaptoethanol. The relative molecular weights of the resolved protein fractions were compared with those of marker proteins. For such purpose, five micrograms of protein per lane was used; gels were fixed and stained with silver stain.

Separated latex proteins (40 μ g per lane) were electrotransferred from the gel to 0.45 nm pore size nitrocellulose membranes (SMI) for 1 hour at 330 mA.²⁵ Membranes were then incubated for 1 hour in a blocking buffer containing 5% horse serum in PBS pH 7.4 and cut into 5-mm strips for immunostaining. All sera from patients and controls, diluted 1:5 v/v in a blocking buffer, were added to each strip and incubated for 12 hours at room temperature.

After washing (0.05% Tween-PBS), an amplifier system was used, whereby strips were first incubated with biotinylated goat antihuman IgE (ϵ -chain specific) antibody (Vector, CA) diluted 1/2000 in blocking buffer (2 hours at 37°C), and then, with ExtrAvidin-alkaline phosphatase conjugate (SIGMA) for 1 hour at 37°C. Strips were washed and subsequently stained with 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium (SIGMA).

Sera

Serum specimens were obtained from 309 subjects aged 1 to 68 years old. They were divided into four groups:

Group I: Sera chosen at random from 28 patients with multiple surgeries: 27 with spina bifida and 1 with a brain tumor; 12 females and 16 males aged 1–24 years (mean age: 7.8). The number of surgeries is included in Table 2.

Group II: Sera chosen at random from 98 health care workers: surgeons, dentists, nurses, operating room nurses, etc.; 78 females and 20 males aged 18–57 years (mean age: 37.7).

Group III: Sera from 123 randomly chosen outpatients treated in the Allergy Department; 78 females and 45 males aged 1–68 years (mean age: 28.3).

Control group: Sera from 60 healthy volunteers with normal total IgE according to age in our population, with only occasional contact with latex, and no latex-associated symptoms; 36 females and 24 males aged 1–68 years (mean age: 31.2).

Skin-Prick Test

In attempts to evaluate the in vivo reactivity of the extract, a total of 10 subjects (5 from group II and 5 from group III) were tested for their skin reactions to latex protein extract on the anterior face of the forearm. They were chosen based on the clinical histories and characteristic signs compatible with latex allergy, but no anaphylactic

reaction after contact with latex-made products. Because our latex extract is not standardized, SPT was not applied to all patients. Patients from group I were not exposed to latex proteins because it is recommended to avoid any contact with latex.

The latex protein extract was diluted 1/10,000 in NaCl 0.9 g/L with 50% glycerol, and was sterilized by filtration through a 0.22 μ m membrane filter. It was stored at 4°C. A glycerol-solution of NaCl 0.9 g/L formed the negative control and histamine dihydrochloride (1 mg/mL), the positive control.

Cutaneous reactions were read after 15 minutes and the mean diameter of the wheal was measured. It was considered positive when the mean diameter of the reaction wheal was at least 3 mm and surrounded by an erythema, as compared with negative control values (wheal < 2 mm and no erythema).

Quantification of Total IgE

Total IgE was determined by ELISA using paper disks activated with cyanogen bromide (CNBr). Disks were coupled with rabbit antihuman IgE (ϵ -chain specific) (Behring) and incubated with sera (diluted 1/10) for 12 hours at room temperature. After adequate washing, they were incubated with monoclonal antihuman IgE (ϵ -chain specific) alkaline phosphatase conjugated 1/5000 (SIGMA) (12 hours at room temperature). Color was developed with *p*-nitrophenyl phosphate disodium, and optical density (OD) was measured at 405 nm.

The calibration curve was obtained using a commercial IgE standard (Behring), and checked against an international standard (Immunoglobulin E Reference Preparation 69/204).

Detection of Specific IgE by EAST

Determination of specific IgE in human sera was performed by using CNBr-activated cellulose disks. Latex proteins extracted in the presence of SDS were coupled to CNBr-activated paper disks at 1 mg/mL concentration in 0.1 M carbonate/bicarbonate buffer, pH 8.5, according to the method of Ceska et al.²⁶ Disks were incubated first for 1 hour in a blocking buffer containing 0.05 M ethanolamine, and then with 50 μ L serum for 12 hours at room temperature. After thorough washing, monoclonal antihuman IgE alkaline phosphatase conjugate 1/5000 (SIGMA) was added for 12 hours at room temperature and *p*-nitrophenyl phosphate (NPP, SIGMA) was used as substrate. OD was measured at 405 nm.

To optimize the coating of paper disks, different protein concentrations of the latex extract were tested. The concentration leading to no further increase of OD when analyzed with a pool of positive sera (1 mg/mL), was chosen as working condition. To determine whether this amount of coupled antigen was sufficient to bind all the antilatel IgE antibodies present in the serum, and to verify that no inter-

ference with specific IgG antibodies was occurring, a pool of positive sera was adsorbed with an antihuman IgG (γ -chain specific) monoclonal antibody and then, specific antilatel IgE was measured as described above.

Assay specificity was controlled using serum samples with specific IgE against other allergens and a total IgE level higher than 200 IU/mL. In addition, a serum with more than 20,000 IU/mL of total IgE was also tested. Background value was determined by analyzing 60 sera from individuals belonging to the control group.

To measure specific IgE in classes, a calibration curve was obtained by using a pool of anti-*Dermatophagoides* sp. sera, as reported by Lundkvist.²⁷

Detection of Specific IgE by ELISA

Indirect ELISA was developed by coating the microtiter plates (NUNC, Maxisorp) with the latex extracts obtained with SDS (1 μ g/well). After blocking with PBS containing 5% horse serum (1 hour at 37°C), wells were incubated with 100 μ L of patient or control sera (diluted 1/5) for 12 hours at 4°C. After washing, monoclonal anti-IgE (ϵ -chain specific) antibodies-alkaline phosphatase conjugate (SIGMA) 1/5000 was added. Color was developed with *p*-nitrophenyl phosphate disodium, and OD was measured at 405 nm.

To determine the cutoff value of the assay, the 60 serum samples from the control group were tested under the same conditions as those used for patient sera. The cutoff value was calculated as the mean optical density plus 3 standard deviation.

To compare the specificity of the test with a commercial kit, ten positive sera for latex-specific IgE antibodies by the ELISA method were also analyzed by the CAP System (Pharmacia Diagnostic, Uppsala, Sweden) by Dr. F. Leynadier (Hospital Rothschild, Paris, France).

RESULTS

Protein Content of Latex Extracts

The C serum obtained by ultracentrifugation was used for the extracts, because this fraction had the highest protein content (5.3 mg/mL). The addition of different detergents increased the amount of proteins extracted. The protein concentration of the different extracts was: 8.8 mg/mL with SDS, 6.1 mg/mL with Triton X100, 7.9 mg/mL with NP40 and 6.0 mg/mL with deoxycholate.

Figure 1 (lane 1), shows the protein profile of the latex extract as determined by SDS-PAGE. Protein bands within a wide range of molecular mass—up to 100 kDa, were detected. The most intense bands were observed in the regions corresponding to 14 and 18. Nevertheless, some other bands could be observed with relative molecular mass of 20, 27, 30, 33, 46, 61, and 90 kDa.

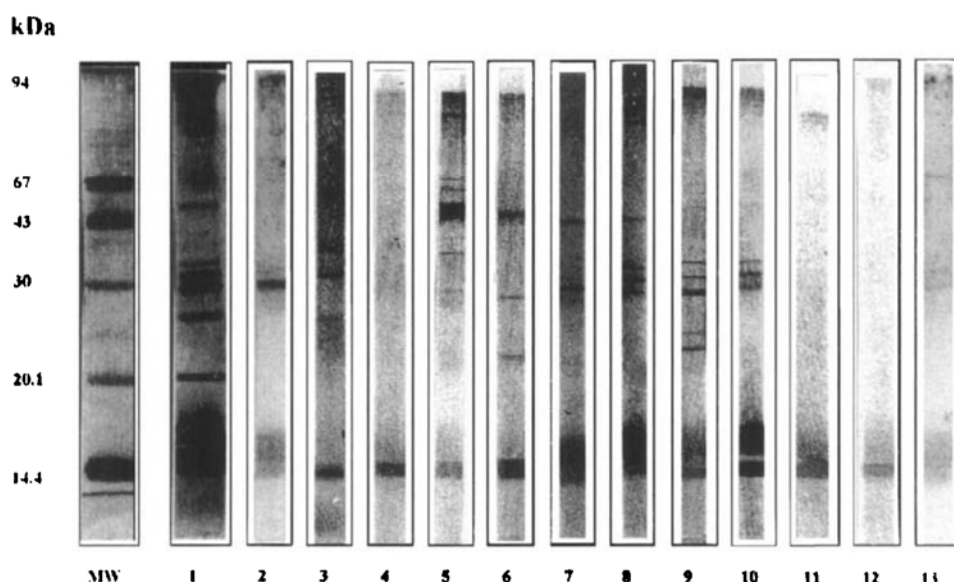


Figure 1. Analysis by SDS-PAGE and immunoblotting. MW: molecular weight markers; 1: SDS-PAGE profile of ammoniated latex protein extract stained with silver stain; 2–13: IgE immunoblots of latex extracts developed with sera from patients belonging to group I (2–5), group II (6–9) and group III (10–13).

Quantification of Specific IgE

Age-related determination of normal total IgE levels in our population were as follows: less than 30 IU/mL up to 3 years old; between 30 and 60 IU/mL from 3 to 6 years old; up to 100 IU/mL, over 6 years old.

Table 1 shows the results of total and specific IgE levels in sera from all subjects analyzed. In group I, 60.7% (17/28) of the sera analyzed showed an elevated total IgE, from which 9 (32.1%) also had specific IgE against latex proteins, as determined by both EAST and ELISA. With regard to group II, 35.7% (35/98) of the sera had an elevated total IgE level, from which 17 (17.3%) showed the presence of specific IgE to latex proteins. From patients assisted at the Allergy Department (group III), 74.8% (92/123) had total IgE higher than the normal value, from which specific IgE could be detected by EAST and ELISA in 23 cases (18.6%). Sera belonging to the control group had neither total IgE level

higher than normal, nor specific IgE against latex proteins, and the clinical history indicated no atopy related to allergy to manufactured latex products.

To check the specificity of the assays, 28 sera from patients found to be atopic to allergens that had not been reported to cross-react with latex proteins (egg, penicillin, sulfamethazazol, *Dermatophagoides*, cat and dog dander) were tested by the three immunoenzymic methods. No specific antilatel protein IgE was detected in any case.

Tables 2 and 3 show the results from the individual analysis of sera from patients with specific IgE of each group. As can be seen, sera of every group showing positive specific IgE by EAST (class 2 to 4) were also positive to ELISA. Moreover, all sera tested showed total IgE levels higher than normal according to age.

The ten sera tested with the CAP System were confirmed to be positive for latex-specific IgE antibodies (Tables 2 and 3).

TABLE I
Total and Specific IgE Antilatel Proteins Results from the Analyzed Sera

Groups*	No. of Patients	Patients with Total IgE Level		Patients with Specific IgE	
		Normal #	Elevated	EAST	ELISA
I	28	11	17 (60.7%)*	9 (32.1%)*	9 (32.1%)
II	98	63	35 (35.7%)	17 (17.3%)	17 (17.3%)
III	123	31	92 (74.8%)	23 (18.6%)	23 (18.6%)
IV	60	60	0	0	0

*Groups: I, patients with multiple surgeries; II, health care workers; III, outpatients of the allergy department; IV, healthy subjects.

#Percentage values are referred to total number of patients of each group.

TABLE II

Data of Patients with Multiple Surgeries (group I)

Patient	Age (years)	Total IgE (IU/mL)	Specific IgE EAST (class)	Specific IgE ELISA (OD ₄₀₅)	Specific IgE CAP System (kIU/mL—class)	No. of Surgeries	Clinical Signs*
1	8	1360	4	1.100	1.18–2	1	IA
2	4	192	4	0.850		3	IA
3	9	116	2	0.551	14.2–3	11	U
4	4	150	2	0.485	2.56–2	2	NS
5	5	118	2	0.610		3	NS
6	12	230	3	0.820		9	PA
7	13	112	2	0.457		3	NS
8	2	130	3	0.801		2	NS
9	1	22	0	0.144		3	NS
10	10	1215	0	0.143		5	NS
11	4	72	0	0.119		3	NS
12	12	23	0	0.120		2	NS
13	5	53	0	0.128		3	NS
14	6	135	0	0.136		2	NS
15	17	62	0	0.144		23	NS
16	1	36	0	0.124		5	NS
17	24	42	0	0.102		15	NS
18	2	25	0	0.120		2	NS
19	14	98	0	0.126		2	NS
20	14	85	0	0.151		4	NS
21	13	153	0	0.162		11	NS
22	5	116	0	0.211		10	NS
23	2	119	0	0.201		2	NS
24	13	26	0	0.198		11	NS
25	6	116	0	0.123		2	NS
26	14	25	0	0.122		4	NS
27	7	95	0	0.151		2	NS
Cutoff value				0.279	1		

*Clinical signs: IA, intrasurgical anaphylaxis; U, urticaria; PA, post-operative asthma; NS, no signs.

Immunoblotting

No IgE-antilatex protein antibodies could be detected when developing the immunoblotting with normal control sera. The binding of latex proteins to IgE antibodies in some patients belonging to the different groups are shown in Fig. 1, lanes 2 to 13. The other sera analyzed showed similar IgE immunoblot pattern. The most intense reaction corresponded to the bands localized in the 14–17-kDa region. Nevertheless, reactivities were observed against several other proteins, especially those with relative molecular mass in the 27–33 and 40–67-kDa regions.

When comparing the patterns of IgE reactivities among the three groups of subjects, it could be observed that, in group I (lanes 2–5), all sera revealed proteins in the regions of 14 and 30–33. However, reactivities against components with relative MW lower than 10 (lane 3), with MW in the region of 27 (lane 3), and in the 30–67 zone (lanes 3 and 5) could be observed. With regard to group II (lanes 6–9), all positive sera had IgE against components with MW in the regions of 14 and 30–33. Moreover, some sera also detected protein fractions with an

MW in the 18 zone (lanes 7, 8, and 9), in the 27 region (lanes 6 and 9), and with higher MW (67, 81, and higher) (lanes 6, 7, and 8). In contrast, all sera belonging to subjects from group III (lanes 10–13) revealed components in the region of 14, whereas some of them detected proteins with higher MW, as well (lanes 10 and 13).

Similar patterns of reactivity were observed working under reducing and nonreducing conditions (data not shown).

Skin-Prick Test

The subjects tested from group II and III (marked in Table 3) exhibited a positive skin reaction with a mean diameter of the wheal greater than 3 mm and erythema. All of them had a history of exposure to latex products and sera from these patients were analyzed by the CAP System for specific IgE.

DISCUSSION

Several extraction procedures from natural latex were assessed to obtain the best yield in protein content. Some authors have reported that a higher amount of protein

TABLE III

Total and Specific IgE Level of Positive Sera of Patients from Groups II and III

Patient	Age (years)	Total IgE (IU/mL)	Specific IgE EAST (class)	Specific IgE ELISA (OD 405)	Specific IgE CAP System (KIU/ml—class)	Clinical Signs
Group II	1	39	116	3	0.807	Anaphylaxis by penicillin
	2*	30	118	2	0.510	1.10–2 Dermatitis caused by gloves
	3	23	112	2	0.861	Angioedema, urticaria
	4*	28	128	3	0.522	2.96–2 Rinitis
	5	18	182	2	0.390	ND#
	6	45	218	3	0.420	ND
	7	39	119	2	0.453	ND
	8	26	128	3	0.406	ND
	9	45	116	3	0.485	ND
	10	22	192	3	0.820	Dermatitis
	11*	30	148	3	1.160	3.70–3 Prurito, urticaria
	12	35	65	2	0.360	Asthma, rinitis
	13	40	450	2	0.367	Dermatitis, angioedema
	14*	24	123	2	0.431	1.55–2 Dermatitis caused by gloves
	15*	22	125	3	0.911	3.73–3 Rinitis, prurito
Group III	1	23	620	2	0.675	Asthma
	2	5	1310	2	0.483	ND
	3	6	1210	2	0.459	Asthma
	4	12	198	2	0.403	Rinitis
	5	35	1840	3	0.516	ND
	6	3	160	2	0.428	Urticaria, angioedema caused by balloons
	7	25	162	2	0.429	Dermatitis caused by gloves
	8	17	173	2	0.430	ND
	9	9	285	3	0.789	Rinitis, asthma
	10	6	243	2	0.652	Rinitis
	11*	27	118	2	0.425	1.86–2 Urticaria, angioedema caused by gloves
Group III	12*	48	236	2	0.418	3.27–2 Prurito, urticaria
	13	42	2140	3	1.130	Asthma
	14	18	1030	3	0.920	ND
	15	18	196	2	0.410	ND
	16*	38	109	3	0.790	3.80–3 Urticaria, angioedema caused by condoms
	17	26	116	3	0.750	Urticaria, angioedema
	18	25	243	2	0.405	Asthma
	19*	20	118	2	0.410	2.41–2 Prurito, urticaria caused by gloves
	20	23	152	2	0.423	Prurito, urticaria caused by gloves
	21	18	285	2	0.398	Generalized urticaria caused by gloves
	22*	50	116	2	0.428	Urticaria caused by banana
	23	21	152	2	0.398	Asthma
Cutoff value				0.279	1	

*Sera from subjects tested by SPT.

#ND: no data.

components could be extracted from nonammoniated natural latex.^{16,28} The use of ammoniated latex may be more convenient, because its manipulation, transport, and storage are easier.⁹ In addition, it is the raw material to manufacture different products in all countries. Consequently, the latex-product allergens are derived from this source. Although the largest amount of the natural rubber latex proteins is found

in the C serum, a considerable amount is tightly bound to the surface of rubber particles as well.²⁹ Use of ionic detergents (mainly SDS) facilitates the extraction of attached proteins.²⁸ Moreover, proteins from subcellular organelles may be found in the sap of *Hevea brasiliensis*, as a result of the presence of ammonia during storage. It has been shown that the lutoid fraction contains allergenic proteins.⁹

Its electrophoretical protein profile, obtained by SDS-PAGE, shows similar fractions as those reported in the literature, with bands mainly in the 14, 17, 20, 27, 30, 33, 38, 47, 67, and 90 kDa zones of molecular mass.^{16,17} When used to detect the presence of specific IgE antibodies in sera from different groups of subjects, we found that patients from group I showed specific IgE antibodies in 32.1% of the cases; all of them had high total IgE levels. Other authors reported the highest prevalence for this group: between 13 and 51% determined by SPT and RAST screening.^{5,16,17,30,31} Nevertheless, there is a report on children with spina bifida in Venezuela, where a low prevalence of 4.3%, associated with a poor socioeconomic condition, was observed.³² In our country, because of socioeconomic factors, the number of surgeries in our patients is not as high as that seen in developed countries; besides, no daily enemas or glove fecal disimpaction were practiced on these patients. This could explain the lower prevalence rate in comparison with that of the international literature.

In the health care workers group (group II), 17.3% of sera had specific IgE antibodies and total IgE levels higher than normal. All the sera with total IgE levels within normal range were negative by EAST or ELISA. For this group, the reported prevalence, as determined by SPT and RAST in Europe,^{3,33} USA, and Canada^{34,35} ranged from 2.8% to 17%.

In the outpatient group assisted at the Allergy Department (group III), 74.8% were atopic and 18.6% of positive sera with specific IgE were detected. All of them had total IgE levels higher than normal. It is worth noting that cross-reactions between latex proteins and allergens from different sources (banana, avocado, tomato, kiwi, peach, melon, peanut, grass, tree, and weed pollen, etc.) have been reported.^{36,37,38} Different authors report prevalence rates ranging from below 1% to 12%, depending on personal atopy.^{3,7,39} Atopy seems to be the principal determinant for latex sensitization after repeated exposures. In our population, sensitization to environmental allergens, such as pollen, is very frequent. This could explain the higher prevalence observed for this group of patients. A group of cytoskeletal proteins, the profilins, constitute an important crossreactive IgE binding component present in several plant sources (pollen, fresh fruits, vegetables, and natural rubber latex). Besides, patients sensitized to profilin were shown to react with profilins of many species.³⁷

In natural latex, these proteins are located in the cytosolic fractions and have a relative MW between 14 and 18. The immunoblots showed a strong and wide band in the 14 zone when revealed with sera from latex-allergic patients. This region could contain not only the REF, but also the cross-reactive profilin. Some reports indicate a prevalence of sensitization to profilin in several populations of between 10% and 30%.⁴⁰ The presence of IgE antibodies to profilin in patients allergic to latex could be the consequence of a sensitization to species other than latex.

Besides, this group includes several children, and there is evidence that incidence of latex allergy in children is higher (19%) than in adults (7%).^{7,38}

All sera showing specific IgE by ELISA were also positive by RAST. Besides, 100% (46/46) of the positive sera showed the presence of IgE reactivity in the region of 14 kDa by immunoblotting. Akasawa et al.²⁸ found that the 23% of latex-allergic subjects reacted by immunoblotting with the 14.6-kDa purified protein REF. By two-dimensional electrophoresis, several polypeptides have been detected within this fraction. Then, this fraction contains not only the REF-protein but also other allergens with the same relative molecular mass, as well.^{5,16,17}

Some positive sera, as determined by EAST, ELISA, and immunoblotting, were also positive when analyzed by using a commercial kit (CAP System). Therefore, the different ways of coating the solid supports with proteins would not alter the immunodominant epitopes present in latex proteins.

In the control group (group IV), sera from subjects with no occupational exposure to latex products and normal total IgE levels according to age, no specific IgE serum antibodies against latex proteins were detected with the tests used.

No correlation was found between the levels of specific IgE and the number of proteins recognized by the sera. Besides, a pattern of allergen recognition characterizing each group could not be detected by immunoblotting, like other studies do. The EAST, ELISA, and immunoblotting assays described seems to be specific, because they were negative with sera of healthy control subjects and from patients atopic to unrelated allergens.

Comparison of our serologic test results with those of prick tests and use tests (CAP System) was carried out in a smaller group of patients from each group. All subjects with signs of atopy after exposure to latex products had serum-specific IgE and positive SPT, thus showing an association between the serology, clinical signs, and an *in vivo* test.

From our results, prevalence observed for patients with multiple surgeries and health care workers are similar to those reported in the literature of other countries. However, atopic patients showed a higher prevalence than that found in literature.

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ASTELIN® (azelastine hydrochloride) Nasal Spray, 137 mcg

For Intranasal Use Only

Brief Summary

The following is a brief summary only. Before prescribing, see complete Prescribing Information in Astelin® Nasal Spray product labeling.

INDICATIONS AND USAGE

Astelin® Nasal Spray is indicated for the treatment of the symptoms of seasonal allergic rhinitis such as rhinorrhea, sneezing, and nasal pruritus in adults and children 12 years and older.

CONTRAINDICATIONS

Astelin® Nasal Spray is contraindicated in patients with a known hypersensitivity to azelastine hydrochloride or any of its components.

PRECAUTIONS

Activities Requiring Mental Alertness: In clinical trials, the occurrence of somnolence has been reported in some patients taking Astelin® Nasal Spray; due caution should therefore be exercised when driving a car or operating potentially dangerous machinery. Concurrent use of Astelin® Nasal Spray with alcohol or other CNS depressants should be avoided because additional reductions in alertness and additional impairment of CNS performance may occur.

Information for Patients: Patients should be instructed to use Astelin® Nasal Spray only as prescribed. For the proper use of the nasal spray and to attain maximum improvement, the patient should read and follow carefully the accompanying patient instructions. Patients should be instructed to prime the delivery system before initial use and after storage for 3 or more days (see PATIENT INSTRUCTIONS FOR USE). Patients should also be instructed to store the bottle upright at room temperature with the pump tightly closed out of the reach of children. In case of accidental ingestion by a young child, seek professional assistance or contact a poison control center immediately.

Patients should be advised against the concurrent use of Astelin® Nasal Spray with other antihistamines without consulting a physician. Patients who are, or may become, pregnant should be told that this product should be used in pregnancy or during lactation only if the potential benefit justifies the potential risks to the fetus or nursing infant. Patients should be advised to assess their individual responses to Astelin® Nasal Spray before engaging in any activity requiring mental alertness, such as driving a car or operating machinery. Patients should be advised that the concurrent use of Astelin® Nasal Spray with alcohol or other CNS depressants may lead to additional reductions in alertness and impairment of CNS performance and should be avoided (see Drug Interactions).

Drug Interactions: Concurrent use of Astelin® Nasal Spray with alcohol or other CNS depressants should be avoided because additional reductions in alertness and additional impairment of CNS performance may occur.

Cimetidine (400 mg twice daily) increased the mean C_{max} and AUC of orally administered azelastine hydrochloride (4 mg twice daily) by approximately 65%. Ranitidine hydrochloride (150 mg twice daily) had no effect on azelastine pharmacokinetics.

Interaction studies investigating the cardiac effects, as measured by the corrected QT interval (QT_c), of concomitantly administered oral azelastine hydrochloride and erythromycin or ketoconazole were conducted. Oral erythromycin (500 mg three times daily for seven days) had no effect on azelastine pharmacokinetics or QT_c based on analyses of serial electrocardiograms. Ketoconazole (200 mg twice daily for 7 days) interfered with the measurement of azelastine plasma concentrations; however, no effects on QT_c were observed.

No significant pharmacokinetic interaction was observed with the coadministration of an oral 4 mg dose of azelastine hydrochloride twice daily and theophylline 300 mg or 400 mg twice daily.

Geriatric Use: U.S. placebo-controlled clinical trials included 11 patients above the age of 60 years who were treated with Astelin® Nasal Spray. While this number is very small and no substantial conclusions can be drawn, the adverse events in this group were similar to patients under age 60 years.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies in rats and mice with oral azelastine hydrochloride for 24 months at doses up to 30 mg/kg/day and 25 mg/kg/day, respectively (240 and 100 times the maximum recommended human daily intranasal dose on a mg/m² basis), revealed no evidence of carcinogenicity.

Azelastine hydrochloride showed no genotoxic effects in the Ames test, DNA repair test, mouse lymphoma forward mutation assay, mouse micronucleus test, or chromosomal aberration test in rat bone marrow.

Reproduction and fertility studies in rats showed no effects on male or female fertility at oral doses of up to 30 mg/kg/day (240 times the maximum recommended human daily intranasal dose on a mg/m² basis). At 68.6 mg/kg/day (550 times the maximum recommended human daily intranasal dose on a mg/m² basis), the duration of estrous cycles was prolonged and copulatory activity and the number of pregnancies were decreased. The numbers of corpora lutea and implantations were decreased; however, the implantation ratio was not affected.

Pregnancy Category C: Azelastine hydrochloride has been shown to be embryotoxic, fetotoxic, and teratogenic (external and skeletal abnormalities) in mice at an oral dose of 68.6 mg/kg/day (280 times the maximum recommended human daily intranasal dose on a mg/m² basis).

At an oral dose of 30 mg/kg/day (240 times the maximum recommended human daily intranasal dose on a mg/m² basis), delayed ossification (undeveloped metacarpus), and the incidence of 14th rib were increased in rats. At 68.6 mg/kg/day (550 times the maximum recommended human daily intranasal dose on a mg/m² basis) azelastine hydrochloride caused abortion and fetotoxic effects in rats. The relevance to humans of these skeletal findings noted at only high drug exposure levels is unknown.

There are no adequate and well-controlled clinical studies in pregnant women. Astelin® Nasal Spray should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether azelastine hydrochloride is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Astelin® Nasal Spray is administered to a nursing woman.

Pediatric Use: Safety and efficacy of Astelin® Nasal Spray in pediatric patients below the age of 12 years have not been established.

ADVERSE REACTIONS

Adverse experience information for Astelin® Nasal Spray is derived from six well-controlled, 2-day to 8-week clinical studies which included 391 patients who received Astelin® Nasal Spray at a dose of



2 sprays per nostril twice daily. In placebo-controlled efficacy trials, the incidence of discontinuation due to adverse reactions in patients receiving Astelin® Nasal Spray was not significantly different from vehicle placebo (2.2% vs 2.8%, respectively).

In these clinical studies, adverse events that occurred statistically significantly more often in patients treated with Astelin® Nasal Spray versus vehicle placebo included bitter taste (19.7% vs 0.6%), somnolence (11.5% vs 5.4%), weight increase (2.0% vs 0%), and myalgia (1.5% vs 0%).

The following adverse events were reported with frequencies ≥2% in the Astelin® Nasal Spray treatment group and more frequently than placebo in short-term (≤2 days) and long-term (2-8 weeks) clinical trials.

ADVERSE EVENT	Astelin® Nasal Spray n = 391	Vehicle Placebo n = 353
Bitter Taste*	19.7	0.6
Headache	14.8	12.7
Somnolence*	11.5	5.4
Nasal Burning	4.1	1.7
Pharyngitis	3.8	2.8
Dry Mouth	2.8	1.7
Paroxysmal Sneezing	3.1	1.1
Nausea	2.8	1.1
Rhinitis	2.3	1.4
Fatigue	2.3	1.4
Dizziness	2.0	1.4
Epistaxis	2.0	1.4
Weight Increase*	2.0	0.0

* P<0.05, Fisher's Exact Test (two-tailed)

The following events were observed infrequently (<2% and exceeding placebo incidence) in patients who received Astelin® Nasal Spray (2 sprays/nostril twice daily) in U.S. clinical trials.

Cardiovascular: flushing, hypertension, tachycardia.

Dermatological: contact dermatitis, eczema, hair and follicle infection, furunculosis.

Digestive: constipation, gastroenteritis, glossitis, ulcerative stomatitis, vomiting, increased SGPT, aphthous stomatitis.

Metabolic and Nutritional: increased appetite.

Musculoskeletal: myalgia, temporomandibular dislocation.

Neurological: hyperkinesia, hypoaesthesia, vertigo.

Psychological: anxiety, depersonalization, depression, nervousness, sleep disorder, thinking abnormal.

Respiratory: bronchospasm, coughing, throat burning, laryngitis.

Special Senses: conjunctivitis, eye abnormality, eye pain, watery eyes, taste loss.

Urogenital: albuminuria, amenorrhea, breast pain, hematuria, increased urinary frequency.

Whole Body: allergic reaction, back pain, herpes simplex, viral infection, malaise, pain in extremities, abdominal pain.

In controlled trials involving nasal and oral azelastine hydrochloride formulations, there were infrequent occurrences of hepatic transaminase elevations. The clinical relevance of these reports has not been established.

In addition, the following spontaneous adverse events have been reported during the marketing of Astelin® Nasal Spray and may have no causal relationship with the drug: anaphylactoid reaction, application site reaction, chest pain, condition aggravated, confusion, diarrhea, dyspnea, facial edema, involuntary muscle contractions, paresthesia, parosmia, pruritus, rash, tolerance, urinary retention, vision abnormal, and xerophthalmia.

OVERDOSAGE

There have been no reported overdoses with Astelin® Nasal Spray. Acute overdose by adults with this dosage form is unlikely to result in clinically significant adverse events, other than increased somnolence, since one bottle of Astelin® Nasal Spray contains 17 mg of azelastine hydrochloride. Clinical studies in adults with single doses of the oral formulation of azelastine hydrochloride (up to 16 mg) have not resulted in increased incidence of serious adverse events. General supportive measures should be employed if overdose occurs. There is no known antidote to Astelin® Nasal Spray. Oral ingestion of antihistamines has the potential to cause serious adverse effects in young children. Accordingly, Astelin® Nasal Spray should be kept out of the reach of children. Oral doses greater than 120 mg/kg (480 times the maximum recommended human daily intranasal dose on a mg/m² basis) produced significant mortality in mice. Responses seen prior to mortality were tremor, convulsions, decreased muscle tone, and salivation. Single doses as high as 10 mg/kg (270 times the maximum recommended human daily intranasal dose on a mg/m² basis) were well tolerated in dogs, but single doses of 20 mg/kg were lethal.

DOSAGE AND ADMINISTRATION

The recommended dose of Astelin® Nasal Spray in adults and children 12 years and older is two sprays per nostril twice daily. Before initial use, the screw cap on the bottle should be replaced with the pump unit and the delivery system should be primed with 4 sprays or until a fine mist appears. When 3 or more days have elapsed since the last use, the pump should be reprimed with 2 sprays or until a fine mist appears.

CAUTION: Avoid spraying in the eyes.

Directions for Use: Illustrated patient instructions for proper use accompany each package of Astelin® Nasal Spray.

HOW SUPPLIED

Astelin® (azelastine hydrochloride) Nasal Spray, 137 mcg (NDC 0037-0241-10) is supplied as a package containing a total of 200 metered sprays in two high-density polyethylene (HDPE) bottles fitted with screw caps. A separate metered-dose spray pump unit and a leaflet of patient instructions are also provided. The spray pump unit is packaged in a polyethylene wrapper and consists of a nasal spray pump fitted with a blue safety clip and a blue plastic dust cover.

Each Astelin® (azelastine hydrochloride) Nasal Spray, 137 mcg, bottle contains 17 mg (1 mg/mL) of azelastine hydrochloride to be used with the supplied metered-dose spray pump unit. Each bottle can deliver 100 metered sprays. Each spray delivers a mean of 0.137 mL solution containing 137 mcg of azelastine hydrochloride.

Manufactured under license from

ASTA Medica AG, Germany

by WALLACE LABORATORIES

Division of Carter-Wallace, Inc.

Cranbury, New Jersey 08512-0181

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Rev. 6/98

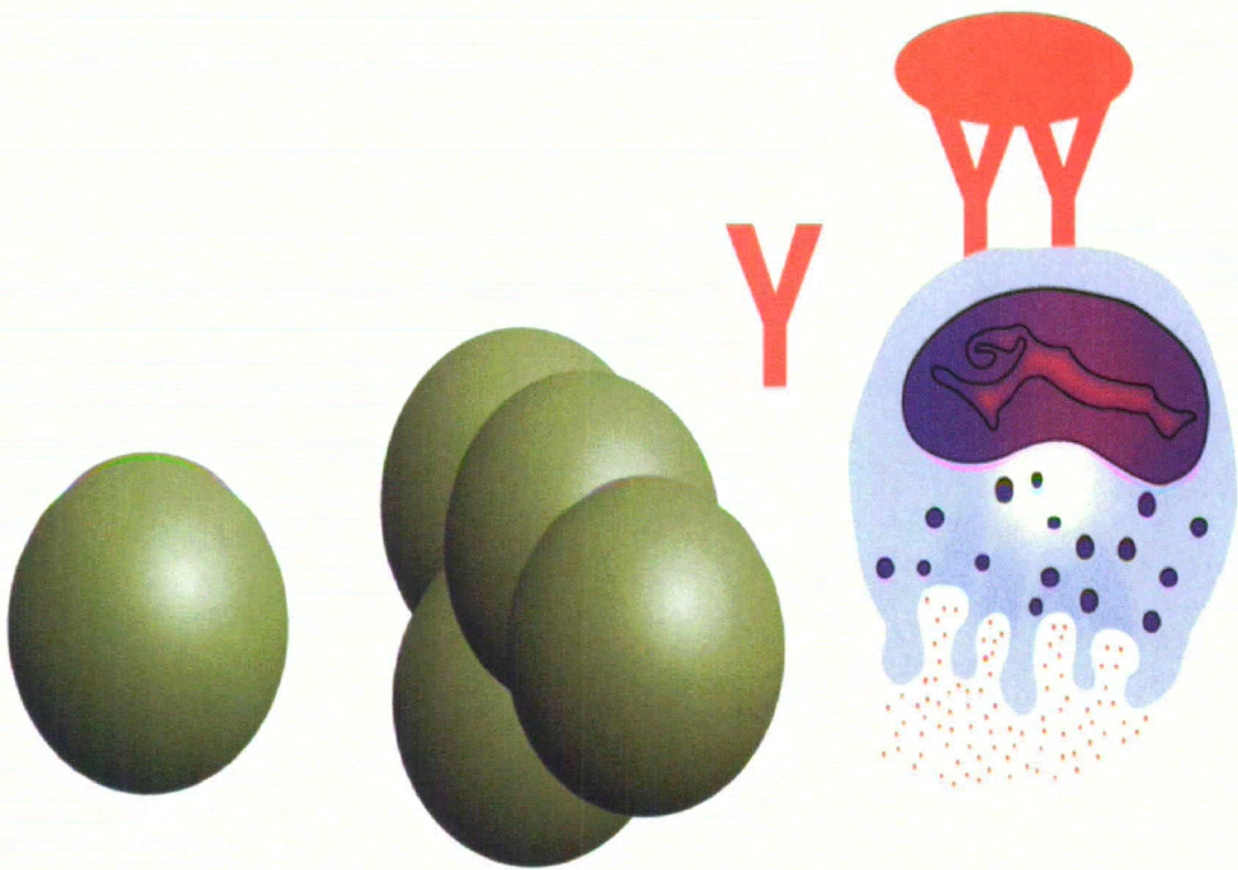
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Cranbury, NJ 08512

ANTI-HISTAMINE AND ANTI-INFLAMMATORY ACTIVITY OF AZELASTINE



...IN VIVO*

Cells	Human Studies
Mast cells	X ¹
Eosinophils	X ²
Neutrophils	X ²

Mediators	Human Studies
Histamine	X ³
Kinins	X ⁴
Leukotrienes	X ⁴
Superoxide free radicals	X ⁵

Adhesion Molecule	Human Studies
ICAM-1	X ²

*The clinical relevance beyond H₁-receptor antagonist activity has not been established.



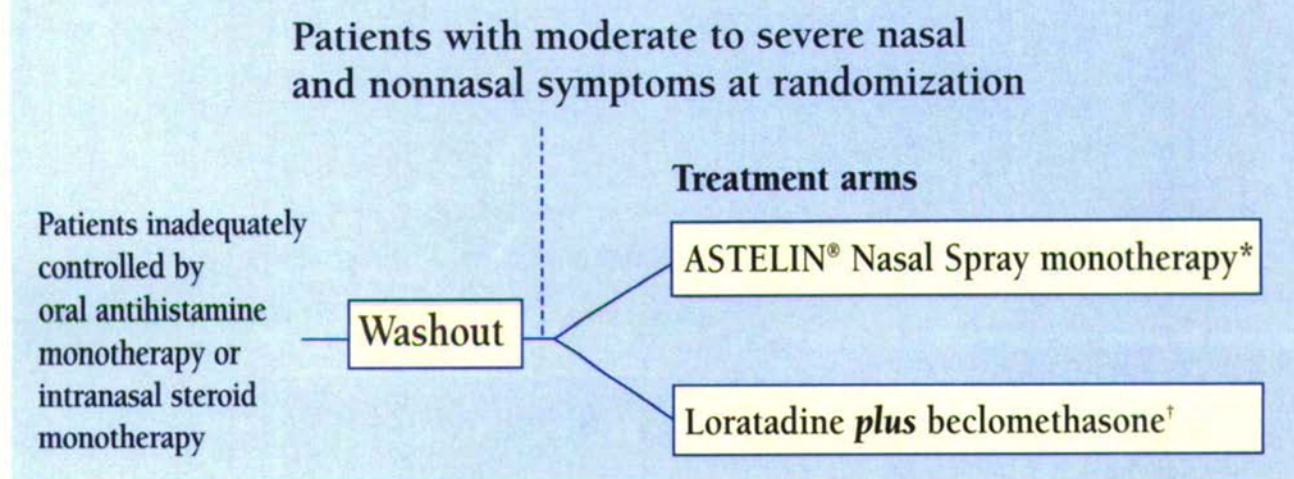
ASTELIN® Nasal Spray monotherapy —

Three well controlled, double-blind, multicenter studies⁶

— N=366 — N=362 — N=342

— 71 sites throughout the United States during spring 1998 allergy season

Study design⁶



*Dosage: Two sprays per nostril BID (137 mcg per spray) plus one placebo each morning for seven days.

†Dosage: One loratadine 10-mg tablet each morning plus beclomethasone two sprays per nostril BID (336 mcg per day) for seven days.

First-Line
Antihistamine
With A
Difference

Astelin
(azelastine HCl)



Nasal
Spray
137 mcg

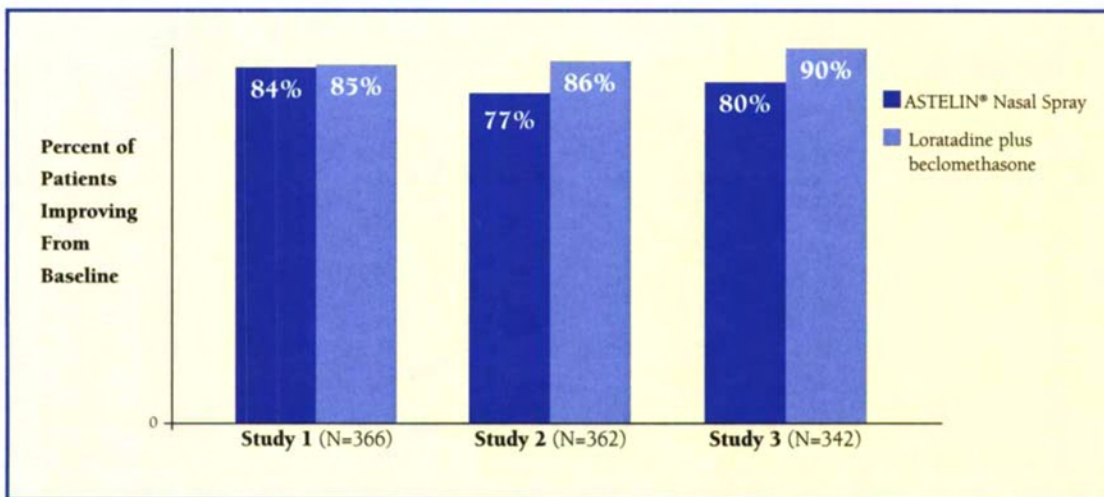


as effective as loratadine *plus* beclomethasone combination therapy⁶

- Patients with moderate to severe symptoms inadequately controlled
- Patient improvement was similar in both treatment arms

Patient response⁶

- No statistically significant differences between treatment arms in physician global evaluation*
- Patient global evaluation†



The most commonly reported adverse events were bitter taste, somnolence, and rhinitis.

*Physician global evaluation of patients' need for additional therapy at end of study.

†There were no statistically significant differences between treatment groups in study 1 and 2; there was a statistically significant difference between treatment groups in study 3 ($P=0.04$).

Please see accompanying brief summary of prescribing information on first page of advertisement.



First-Line
Antihistamine
With A
Difference

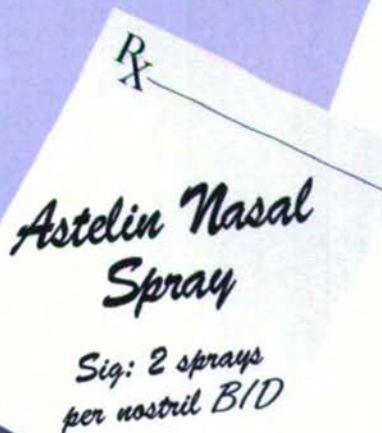
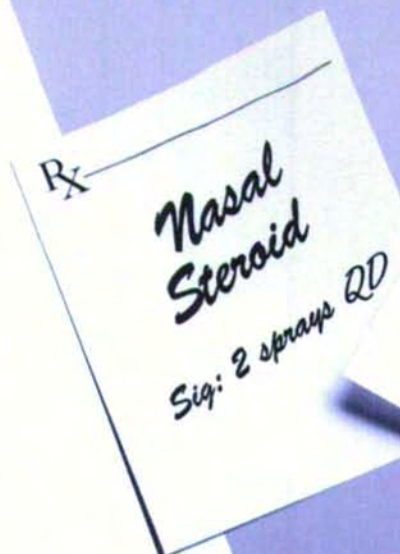
Astelin
(azelastine HCl)

Nasal
Spray
137 mcg

FOR SEASONAL ALLERGIC RHINITIS
IN FIRST-LINE MONOTHERAPY...



AND TO COMPLEMENT
INTRANASAL STEROID THERAPY...



First-Line
Antihistamine
With A
Difference

Astelin
(azelastine HCl)

Nasal
Spray
137 mcg



Please see brief summary of full prescribing information on first page of advertisement.

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