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**Z. Szépfalusi · I. Nentwich · M. Dobner · K. Pillwein**
**R. Urbanek**

# IgE-mediated allergic reaction to hyaluronidase in paediatric oncological patients

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**Abstract** Hyaluronidase has been gaining increasing interest as a spreading factor for better penetration of chemotherapeutics into CNS tumours. Five out of 16 patients with CNS tumours treated with hyaluronidase in addition to chemotherapeutic agents developed symptoms of immediate type allergic reactions, therefore we sought to characterize the harmful allergenic proteins of the bovine testes hyaluronidase enzyme preparation (Neopermease). The role of specific IgE for the allergic reaction was investigated. Using an immunoblotting technique, we investigated sera from 16 children treated with Neopermease (5 of them having developed anaphylactic reactions), 5 patients with atopy (atopic eczema) with high total IgE levels and 4 healthy children. SDS-PAGE of hyaluronidase preparation Neopermease revealed two major bands at 73 and 41–43 kDa. In all 5 sera from patients with adverse reactions, binding of specific IgE antibodies to the 73 and 41–43 kDa bands was found. Two patients reacted with the 73 kDa band exclusively, two patients reacted with both bands, one patient displayed IgE only to the 41–43 kDa band. A specific inhibition of IgE-binding to both bands was achieved after preincubation of the sera in four out of five patients with partially purified bovine hyaluronidase. Furthermore preincubation with gelatin, a stabilising agent in the commercial extract, led to a partial inhibition in the sera of three patients. No specific IgE binding was detected either in the sera of atopic patients, or in the control group.

**Conclusion** IgE mediated allergic reactions to hyaluronidase may occur in paediatric oncological patients treated with hyaluronidase. Whether these children are sensitized by intravenous hyaluronidase treatment or by cross-reactivity of other preformed IgE antibodies, yet to be specified, remains to be elucidated.

**Key words** Hyaluronidase · Allergy IgE-antibodies · Brain tumour

**Abbreviations** *MW* molecular weight · *SDS-PAGE* sodium dodecyl sulphate polyacrylamide gel electrophoresis

## Introduction

Hyaluronidases constitute a group of enzymes with tissue spreading activity [10]. It is found in bee, hornet and ant venom [6, 7, 11], invasive nematodes [8], mammalian (including bovine) testes [3, 6], group B streptococci [15], and in ox spleen, iris and cornea [13]. It could be shown that the hyaluronidases from bee and hornet venom and the PH-20 hyaluronidase present on mammalian spermatozoa are homologous proteins [6, 11]. Cloned white face hornet venom hyaluronidase Dol m 2, a protein of 331 residues, has 56% and 27% sequence identity with honey bee venom allergen hyaluronidase and with PH-20, respectively [11].

Hyaluronidase lessens the normal high viscosity and lubricating quality of hyaluronic acid, a constituent of the extracellular matrix and this improves the diffusion of chemotherapeutic drugs into malignant tissue. Hyaluronidase has also been employed to improve the spread of local anaesthetic solutions through tissue [22].

Brain tumours contain large amounts of hyaluronic acid [4] and the beneficial effect of hyaluronidase for penetration of drugs into tumour cells has been documented [9, 12, 19]. Therefore, hyaluronidase has gained interest as a potential spreading factor during chemotherapy in CNS tumours in children and infants.

Z. Szépfalusi (✉) · I. Nentwich · M. Dobner · K. Pillwein
R. Urbanek
Department of Paediatrics, University of Vienna-AKH,
Währinger Gürtel 18-20, A-1090 Vienna, Austria
Fax: 0043-1-40400-3189

A few cases of allergic reactions following hyaluronidase administration have been recorded, and hyaluronidase appears to have a role in the massive increase in capillary permeability seen in anaphylactic reactions [14].

We have used hyaluronidase since 1992 in the treatment of malignant brain tumours (ependymoma, medulloblastoma, anaplastic astrocytoma, primitive neuroectodermal tumours, pinealoblastoma, neurocytoma and glioblastoma). Anaphylactic reactions during hyaluronidase infusion were recorded in 5 out of 16 patients.

The aim of the study was to characterise the allergenic components in the hyaluronidase using sera from patients who developed immediate type reactions following hyaluronidase administration. The role of specific IgE antibodies was evaluated by inhibition assays.

## Materials and methods

### Patients

Sixteen children suffering from malignant tumours and receiving chemotherapy plus hyaluronidase were studied. All patients received chemotherapeutic drugs in combination with 200,000 international units of hyaluronidase on each day of a treatment. Most of the patients were enrolled in the HIT 91 protocol of the GPO study group for medulloblastoma, pinealoblastoma, supratentorial primitive neuro-ectodermal tumour, neurocytoma, ependymoma and glioblastoma. All patients received dexamethasone 0.15 mg/kg per day intravenously as brain oedema prophylaxis during the course of chemotherapy. Patient data of total IgE and adverse reactions to hyaluronidase are delineated in Table 1. In all patients the symptoms appeared during the i.v.-administration of hyaluronidase. Most of the patients described first the appearance of a burning on the skin, which continued to urticaria or a macular exanthema. Finally, after the stopping of the infusion 3 patients suffered from dyspnoea, 2 patients had additionally blood pressure drop. The cumulative doses of hyaluronidase reached at the time of allergic reaction were 700,000 / 2,400,000 / 3,000,000 / 1,600,000 / 800,000 IU, respectively. Patients 1 and 5 reacted to hyaluronidase at the beginning of the second chemotherapy course, approximatly 2 weeks after the first exposure. Patients 2, 3 and 4 reacted at the $4^{th}$, $5^{th}$ and $3^{rd}$ chemotherapy course, respectively. A control group I (age range from 3 to 9 years) consisted of five patients with atopic disease (atopic eczema, allergic rhinitis, allergic asthma) with high total IgE levels (590 to > 1000 kU/l). Control group II consisted of five healthy children (age range from 2 to 8 years) with normal age-related total IgE levels (0 to 16 kU/l). No symptoms of allergy were reported in this group. None of the control individuals had been treated with Neopermease intravenously. A previous contact with hyaluronidase derived from honey bee venom toxin or streptococcal infections could not be excluded. Skin prick test was not performed because of steroid application and resulting uncertainty of skin tests.

### Drug and enzymes

Commercially available bovine testes derived hyaluronidase (dry powder - Neopermease) was purchased from Sandoz-Sanabo, Vienna, Austria. It was dissolved in double-distilled water to obtain a stock solution of 4 mg/ml concentration. Lyophilised powder of hyaluronidase I-S, IV-S and VIII-S from bovine testes (Sigma, St. Louis, MO, USA) as well as gelatin (Merck, Darmstadt, Germany) were dissolved in redistilled water to obtain a final concentration of 10 mg/ml. Gelatin constituted the stabilizing component of the commercially available bovine testes hyaluronidase. For the immunoblot and inhibition experiments, hyaluronidase I-S was used because of the closest similarity to molecular weight bands displayed by Neopermease. All stock solutions were stored at –20°C.

### Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Molecular weight profile of Neopermease, partially purified bovine testes hyaluronidase (I-S) and gelatin were assessed using a modified method of discontinuous tricine SDS-PAGE (Pharmacia Biotechnology, Uppsala, Sweden). A 15% polyacrylamide gel was used to resolve molecular weight (MW)s between 4 and 200 kDa. Drug and enzyme preparation, Coomassie blue staining and immunoblotting were performed as described previously [5, 20]. Briefly, proteins separated by SDS-PAGE were transferred to nitrocellulose. Nitrocellulose sheets were cut into 0.5 cm strips and incubated for 30 min at room temperature in phophate buffer (pH 7.5) containing 0.5% bovine serum albumin and 0.05% sodium azide. Strips were then incubated overnight at 4°C with patients' sera, diluted 1:10 in above mentioned buffer. After washing, bound IgE was detected by iodine 125-labelled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden) diluted 1:10 in buffer. Autoradiography was performed with intensifying screens (Kodak, Heidelberg, Germany).

MW distribution was determined by densitometric detection (Eliscript 400, Hirschmannn, Germany) using MW markers (Rainbow marker, Amersham, Bucks, England).

### Inhibition experiments

Inhibition experiments were performed as published [20]. Briefly, each serum was preincubated with hyaluronidase I-S at 0.5 mg/ml, gelatin at 0.5 mg/ml at 4°C, overnight. Serum without preincubation served as the positive control. In previous experiments, inhibition with Neopermease lead to complete blocking of IgE antibodies; therefore hyaluronidase I-S was used as the main protein component of Neopermease. Unfortunatly recombinant bovine testes hyaluronidase was not available.

## Results

### SDS-PAGE

Hyaluronidase proteins separated by 15% SDS-PAGE and stained with Coomassie brilliant blue R, revealed two major bands at MW 73 and 41–43 kDa, with some minor bands at MW 50, 33 and 17 kDa (Fig. 1). The 41–43 kDa protein has been shown to represent the major band of human serum, white face hornet and bee venom hyaluronidase [1, 6, 11] under reducing conditions. Under non-reducing conditions, the enzyme preparation appeared to migrate more slowly, with an apparent MW of 72–73 kDa [1].

Hyaluronidase fractions representing different activity grades revealed bands at 73, 50, 41–43, 27, 17 and 13 kDa. Densitometric analysis showed that the main bands of Neopermease, derived from bovine testicular extract corresponds to the main fractions in the hyaluronidase I-S preparation (not shown). Neopermease and hyaluronidase I-S differed only in terms of the quantitative distribution of the bands.

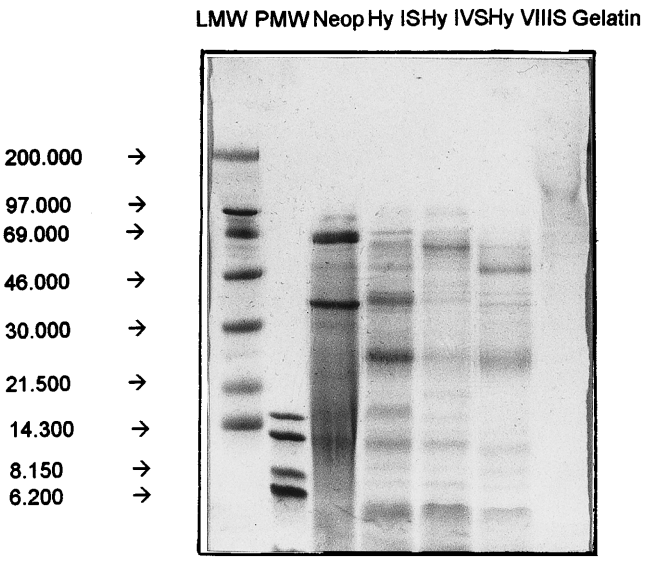



**Fig. 1** Commassie brilliant blue R stained 15% SDS-PAGE shows the separation of Neopermease, hyaluronidase (*Hy-I-S*), hyaluronidase *(Hy IV-S),* hyaluronidase (*Hy VIII-S)* and *gelatin.* Lane *LMW* and *PMW* represent low molecular weight markers and peptide molecular weight markers, respectively

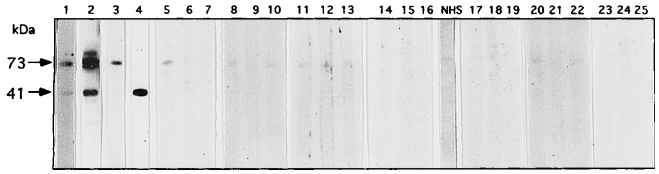



**Fig. 2** IgE immunoblots with sera from 16 patients treated with hyaluronidase (*1–16*), 5 patients with atopy and high total IgE levels (*17–21*) and 4 individuals without previous contact with hyaluronidase (*22–25*). Lane *NHS* represents hyaluronidase-coated strips incubated with normal human serum. Patients 1–5 recognize bands at MW of 73 and 41–43 kDa. Patients 1 and 2 recognize additionally a band at 100 kDa

## Total IgE in hyaluronidase-reactive patients

Four of 5 patients with anaphylactic reactions after Neopermease, but only 1 out of 11 non-reactive patients displayed high total IgE levels (Table 1).

## IgE immunoblots

### *IgE reactivity of sera from patients treated with hyaluronidase*

From 16 hyaluronidase treated patients, 5 (31.2%) displayed IgE binding to the hyaluronidase bands at 41–43 and 73 kDa (Fig. 2). Two patients (3 and 5) reacted with the 73 kDa band exclusively, patient 4 reacted with the 41–43 kDa band exclusively, patients 1 and 2 displayed IgE to the 41–43 and 73 kDa bands. Additionally, patient 2 had IgE binding to the 14, 35 and 100 kDa bands. Total IgE levels were 446, 30, 502, > 1000 and 210 KU/I, respectively. Patient 4 suffered from an IgE-mediated allergic rhinitis with elevated specific IgE levels to Timothy grass and egg white. The remaining 4 patients had no disease in terms of atopy.

### *IgE reactivity of sera from atopic patients and healthy children with hyaluronidase*

With hyaluronidase extract, none of the control patients with atopic disease and high total IgE levels displayed IgE reactivity.

Control sera from children without history of allergy and low total IgE levels showed no IgE reactivity with any hyaluronidase proteins.

## Inhibition experiments

Preincubation of patients sera with hyaluronidase I-S and bovine testicular hyaluronidase (Neopermease) at a

**Table 1** Patients' data. Patients represent individuals treated with hyaluronidase intravenously; numbers 1–5 with allergic reactions upon hyaluronidase, 6–16 without allergic reactions

| Patient | Age, Gender | Diagnosis | Total lgE (kU/L) | Symptoms | Onset |
|---|---|---|---|---|---|
| 1 | 4a, m | Ependymoma grade III | 446 | urticaria, tachycardia, shock | immediate |
| 2 | 5a, f | Medulloblastoma | 30 | tachycardia, exanthema, dyspnoe | immediate |
| 3 | 8a, m | Medulloblastoma | 502 | itching, exanthema, dyspnoe | immediate |
| 4 | 14a, m | Medulloblastoma | >1000 | total body urticaria | immediate |
| 5 | 11a, f | Ependymoma grade II | 210 | dyspnoe, exanthema, shock | immediate |
| 6 | 9a, f | PNET, supratent. | 279 | | |
| 7 | 3a, m | Medulloblastoma | 18 | | |
| 8 | 14a, f | Pinealoblastoma | 37 | | |
| 9 | 7a, f | Medulloblastoma | 3 | | |
| 10 | 8a, f | Choroid plexus papilloma | 9 | | |
| 11 | 16a, f | Medulloblastoma | 30 | | |
| 12 | 6a, f | Medulloblastoma | 38 | | |
| 13 | 5a, m | Astrocytoma grade I | 3 | | |
| 14 | 12a, m | Medulloblastoma | 70 | | |
| 15 | 1.5a, f | Ependymoma grade II | 29 | | |
| 16 | 4a, f | PNET | 13 | | |

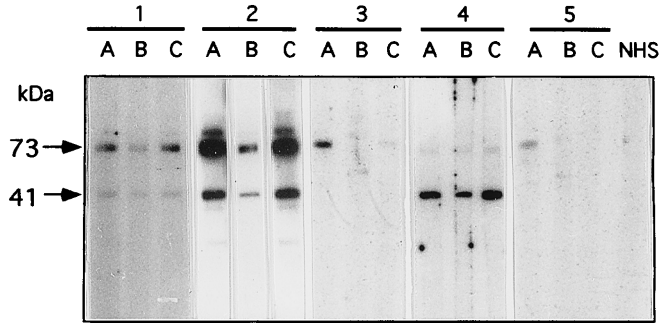



**Fig. 3** Inhibition experiments. Inhibition was performed by preincubation of hyaluronidase reactive sera ($n = 5$) with *A* culture medium (negative control) *B* 0.5 mg/ml hyaluronidase (postive control) and *C* 0.5 mg/ml gelatin overnight at 4°C. Sera were subsequently tested on hyaluronidase extract-coated strips. Lane *NHS* represents hyaluronidase-coated strips incubated with normal human serum

concentration of 0.5 mg/ml was sufficient to strongly reduce IgE binding to hyaluronidase proteins. Both IgE binding to bands at 41–43 and 73 kDa were blunted or inhibited (Fig. 3).

Preincubation of patients sera with gelatin at a concentration of 0.5 mg/ml lead also to partial reduction of IgE binding to hyaluronidase proteins. In patients 3 and 5 strong inhibition of IgE binding to the 73 kDa band could be achieved. For patients 1, 2 and 4 almost no inhibition was detectable (Fig. 3).

As gelatin allergy is only rarely reported in the literature [16], it was assumed that the inhibition with gelatin at the rather high concentration of 0.5 mg/ml might be due to unspecific blocking effects.

## Discussion

The principle findings from this study can be summarized as follows. First, hyaluronidase-specific IgE antibodies were found in five patients with clinical reactions to Neopermease. The specificity of the IgE binding to hyaluronidase could be shown by immunoblotting inhibition experiments. Second, none of the control patients (atopic patients and age-matched individuals) displayed hyaluronidase-specific IgE antibodies. Third, the capacity to mount an IgE antibody reaction to hyaluronidase was not related to the state of individual allergic history. In only one patient, allergic rhinitis was present before beginning chemotherapy. It is therefore concluded that hyaluronidase applied intravenously at doses of 100,000 IU/d bears a strong allergenic potency which might lead to sensitization and subsequent IgE antibody production.

Allergic responses to hyaluronidase have already been documented [17]. Intradermal application in the rat increased capillary permeability of dermal vessels. Hyaluronidase allergy simulating expulsive choroidal haemorrhage in cataract surgery has also been reported [14]. In an earlier case with similar clinical findings no skin reactivity was documented [21]. To our knowledge, this study represents the first description of intravenous application of hyaluronidase with subsequent development of allergic reactions and hyaluronidase-specific IgE. The presence of specific IgE antibodies was accompanied by high total IgE in most of the patients, 446, 30, 502, >1000, 210 kU/l (Table 1), suggesting a strong stimulation of antibody-production. As in patients 3 and 5, IgE binding to hyaluronidase was also inhibited by preincubation with gelatin, the possibility of IgE reactivity towards gelatin must be considered. However, since none of the sera reacted previously with gelatin in immunoblots, the blocking effect in the inhibition experiments might be due to unspecific blocking of IgE-binding sites on hyaluronidase. In the literature, there are only a few reports on IgE reactivity towards gelatin, especially in children with immediate-type reactions to measles and mumps vaccines [16].

Three patients suffered from medulloblastoma, two from ependymoma grade II-III at time of diagnosis, suggesting no association with one specific tumour type. The cumulative doses of hyaluronidase reached at the time of allergic reaction were 700,000 / 2,400,000 / 3,000,000 / 1,600,000 / 800,000 IU, respectively, suggesting no correlation with defined amounts of administered antigen or with a defined time delay until first allergic reaction appears. Therefore, we believe that the IgE production is confined to the high allergenic potency of the hyaluronidase preparation or the stimulatory effect of the co-administration of corticosteroids.

Of particular interest was the fact that the type I immune reaction occurred in an immuno-compromised state receiving dexamethasone (0.15 mg/kg/day i.v.) and high-dose chemotherapy. Part of the immunosuppressive mechanism of glucocorticoids has been elucidated [2, 18]. On the other hand orally applied corticosteroids have been shown to trigger the production of total IgE in patients with allergic diseases [23]. The mechanism which produces the hyaluronidase specific IgE apparently overcomes the inhibitory effects of steroids on the effector phase of the immune response as well as the suppressive effects of chemotherapeutics on T-cell priming and sensitization with antigens.

We conclude that either hyaluronidase represents a potent antigen or that the co-administration of hyaluronidase, chemotherpeutics and dexamethasone triggers IgE synthesis. The latter hypothesis evolves from the observation that hyaluronidase administered as a spreading factor for anesthesic agents was not associated with allergic reactions.

The initiation mechanism of the hyaluronidase-specific IgE production is not known. Possibly the five patients with anaphylactic reactions had already preformed IgE antibodies. We are currently monitoring children with newly diagnosed brain tumours enrolled in the same chemotherapy protocol to find total and hyaluronidase-specific IgE before and during the theraphy.

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