

4th C1 Inhibitor Deficiency Workshop

Budapest 29 April – 1 May 2005

Organized by

- European C1-INH Deficiency Working Group
- Hungarian Society for Immunology
- Foundation for the Prevention and Treatment of Fatal Angio-oedematous Diseases
- Welcome to Hungaria

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Venue & dates

The 4th C1 Inhibitor Deficiency Workshop will take place at:

Hotel Ibis Budapest, Budapest (Hungary)

between 29 April and 1 May, 2005

Adress: Hotel Ibis Budapest,
H-1134 Budapest, Dózsa György út 65.

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Dear Friends,

Hungary has the opportunity to accommodate the meeting of HAE experts for the fourth time in a row, and this is an achievement to be proud of.

Looking back one might ask ‘What was the yield of this series of events?’ The fact that a forum has been devoted to such a rare, but potentially life-threatening disorder is a substantial step forward in itself. It is also a great pleasure that gives us impetus to carry on the tradition of organizing the workshop. The team forged during these meetings held every other year; i.e. the ‘Working Group’ instigated a number of collaborative projects, which would have been unfeasible previously. Currently, research is being supported by a multicenter grant awarded by the European Union three years earlier. Another milestone is the launch of the first international HAE postgraduate course, which was held last year in the picturesque town of Gargnano. The pharma industry has also gained impetus for development and by now, three innovative products have entered the phase of clinical testing. Furthermore, Hereditary Angioedema International (HAEI – the international organization of afflicted patients) has been established. The JACI supplement, a summary of our current knowledge on HAE is the first resource compiled through international effort. The consensus of European and overseas experts has materialized in drafting the Canadian HAE Guideline. Although the foregoing may be regarded as great accomplishments, it is our conviction that there are regions where a lot is to be done yet. The majority of HAE patients are deprived of access to appropriate care world-wide. Essentially, this is related to unfamiliarity with this disorder. In addition to organizing conferences regularly, we have taken the initiative to foster the establishment of national HAE Centers in these countries. This project of utmost importance is now being extended to further countries. Accordingly, we are delighted to welcome at this year’s meeting the delegates

from these newly recruited countries, i.e. Ukraine, Bulgaria, Macedonia, and Romania. It is to be hoped that attendance at the 2007 Workshop will be further expanded by colleagues from other regions of the World.

This year, colleagues from overseas countries have accepted our invitation to expound their views and experience. This reflects appreciation of the high professional level established by previous workshops and naturally, it is also great honor to us.

Finally, we would like to express our thanks to the delegates for attending this conference. Wishing celebrated lectures, meaningful discussions, and fruitful collaboration to all participants of the 2005 HAE Workshop,

Sincerely,

George Füst

President of the Local Committee

Lilian Varga

Secretary of the Local Committee

Henriette Farkas

Secretary of the Local Committee

Friday, 29 April

16:30-17:00 Welcome coffee

17:00-20:30 Opening Ceremony

Chairpersons: *H. Farkas, M. Cicardi*

Greeting of guests

George Füst

Henriette Farkas

Lilian Varga

George Harmat

Sand Animation Performance

by *Ferenc Cako*

„For HAE Patients” Award

to *George Füst*

presented by *Lilian Varga*

Opening lecture

*George Füst: Past, Present and Future of Complement
Laboratories*

Invited experts’ lectures

- *M.M. Frank, K. Baig, P.A. Anderson, S. Hanish, H. Jiang,
D. Craig, Jr. G. Quick, R. Nassar, J. Jagers:*
C1 Inhibitor improves outcome in neonatal
cardiopulmonary bypass
- *A.E. Davis III, C. Cramer, S. Cai, D. Liu:*
Anti-inflammatory activities of C1 inhibitor that are
independent of protease inhibitor
- *B.L. Zuraw, J Herschbach:*
Molecular correction of C1 inhibitor genomic DNA

20:30 Welcome Dinner

Saturday, 30 April

08:00-9:20 Genetics

Chairpersons: *T. Förster, C. Druet*

1. *E. Pappalardo, S. Caccia, L. Maggioni, L.C. Zingale B. Cicardi and M. Cicardi:*
Identification of variables causing different clinical expression of hereditary angioedema
2. *S. Caccia E. Pappalardo, L. Maggioni, M. Cicardi:*
Effect of mutation within the coding region of *C1-INH* gene on protein function in families with HAE
3. *O. Roche, A. Blanch, G. Fontán, M. López-Trascasa:*
Characterization of splicing mutations and identification of possible exonic splicing enhancer in the *C1-INH*
4. *N. Monnier, D. Ponard, M. Abbal, D. Vervloet, C. Drouet:*
Characterization of a mutation of the *C1-INH* gene at splice sequence and associated with HAE-I
5. *T. Förster, W. Kreuz, I. Martinez-Saguer, E. Aygören-Pürsün, K. Bork, CR. Müller, J. Oldenburg:*
Mutation analysis of patients with Hereditary Angioedema in Germany
6. *A. Tordai, T. Hegedûs, H. Farkas, L. Kalmár:*
HAEdb: a novel interactive, locus-specific mutation database of the C1 inhibitor gene
7. *H. Ren, H. Zhang:*
Identification of gene mutations of one Chinese type II HAE family

09:20-09:35 Coffee break

09:35-11:10 Laboratory and clinical management of C1-INH Deficiency

Chairpersons: *M. Frank, E. W. Nielsen*

1. *L.O. Uttenthal, P. Jensen, R. Hald, N.R. Caterer:*
Enzyme-linked immunosorbent assay (ELISA) of functional and total C1-esterase inhibitor (C1-INH)
2. *B. Bilo, G. Porebski, K. Obtulowicz, M. Kapusta, P. Obtulowicz:*
The C4 serum level in Polish patients with hereditary angioedema
3. *M.D. Tarzi, A. Hickey, T. Förster, H.J. Longhurst:*
Normal complement C4 does not exclude hereditary angioedema (HAE)
4. *L. Varga, G. Széplaki, M. Kókai, J. Laki, J. Wieslander, M. Daha, P. Garred, H.O. Madsen, É. Németh, R. Felvinci, B. Visy, G. Harmat, G. Füst, H. Farkas:*
Total lectin pathway (LP) activity of complement is depressed in hereditary angioedema (HAE)
5. *K. Madalinski, M. Cedzyński, A. Cwierzko, E. Nowicka, K. Obtulowicz, H. Gregorek, K. Dzierżanowska-Fangrat:*
Do the mannan-binding lectin pathway of complement activation and some infection contribute to the pathogenesis of hereditary angioedema?
6. *M-E. Moreau, J. Robillard, P. Ponard, L. Bouillet, M. López-Trascasa, A. Adam, C. Drouet:*
Metallopeptidase activity and kinin metabolism in hereditary angioedema (HAE): effect of androgen prophylaxis

Saturday, 30 April – continued

7. *M. Pedrosa, M.T. Caballero, A. Oliveira, M. Bulnes, J. Segura, M.C. López-Serrano:*
Usefulness of abdominal ultrasound in the follow-up of patients with hereditary C1-inhibitor deficiency
8. *R. Felvinci, B. Visy, L. Jakab, H. Farkas:*
The effect of sexual hormone alterations on the frequency of oedematous attacks in patients with HAE

11:10-11:20 Break

11:20-12:05 Poster section (Case Reports / Varia)

Chairpersons: *A. Davis, W. Kreuz*

1. *T. Förster, W. Kreuz, I. Martinez-Saguer, E. Aygören-Pürsün, J Oldenburg, CR Müller:*
Recombinant expression of C1-Inhibitor mutants confirms causality of missense for Hereditary Angioedema - Poster presentation
2. *L. Bouillet, D. Ponard, C. Drouet:*
A HAE –III case associated with C1-INH cleavage without serpin-protease association
3. *B. Piatkowska-Jakubas, A.B. Skotnicki, D. Hawrylecka, P. Mensah, G. Porebski, K. Obtulowicz, B. Bilo, A. Kwapinska:*
C1-inhibitor therapy is effective in capillary leak syndrome due to chemotherapy - case report
4. *E. Aygören-Pürsün, I. Martinez Saguer, E. Rusicke, T. Klingebiel, W. Kreuz:*
Course of two concomitant acute angioedema attacks in a in HAE-Patient treated with s.c. Icatibant

Saturday, 30 April – continued

5. *D. Zabolotny, L. Zabrodskaja, I. Gogunskaja:*
N.M. the first patient with hereditary angioedema from Ukraine
6. *H. Ren, H. Zhang:*
Case report: A case of hereditary angioedema with abdominal pain and ascites
7. *B. Visy, K. Nagy, É. Németh, G. Harmat, L. Varga, H. Farkas:*
Haematologic adverse reactions during danazol treatment
8. *G. Széplaki, L. Varga, Sz. Valentin, M. Kleiber, I. Karádi, L. Romics, G. Füst, H. Farkas:*
Adverse effects of long-term danazol prophylaxis on the lipid profiles of patients with hereditary angioedema: a possible risk factor for atherosclerosis
9. *R. Perricone, M.D. Guarino, S. Guarino, E. Giardina, S. Gambardella, C. De Carolis, C. Perricone, L. Fontana, G. Novelli:*
Germinal mosaicism in HAE
10. *R. Perricone, D. De Nardo, M. Noia, M.D. Guarino, S. Guarino, C. De Carolis, C. Perricone, L. Fontana:*
Successful IVIg treatment of angioedema attacks in a patient with acquired C1-INH deficiency and IgG deficiency

12:05-12:45 Quality of life

Chairpersons: *T. Caballero, A. Price*

1. *N. Prior, E. Remor, M.C. López-Serrano, T. González Quevedo, S. Cimbollek, T. Caballero:*
Health-related quality of life in adults with hereditary angioedema: development of a disease specific questionnaire in Spain (phase I)

Saturday, 30 April – continued

2. *E. Rusicke, E. Aygören-Pürsün, I. Martinez-Saguer, W. Kreuz:*
Improvement of clinical course in Hereditary Angioedema following modification of life style
3. *É. Németh B. Visy, H. Farkas:*
Psychiatric disorders in Hungarian patients with hereditary angioangioneurotic edema

12:45-14:00 Lunch break

14:00-15:45 HAE Centers

Chairpersons: *H. Farkas, Y. Mykal*

1. *H. Farkas, G. Füst, L. Varga:*
Mission is possible
2. *D. Zabolotny, I. Gogunska, L. Zabrodskaja:*
Start of the program to study hereditary angioedema (HAE) in Ukraine
3. *R. Stefanov, P. Krastev, M. Stefanova:*
HAE patients healthcare and management in Bulgaria – history, present and future
4. *K. Stavric, S. Peova, K. Mironska, L. Kareva, S. Nikolovska, Lj. Pavlova, M. Spirovski:*
Diagnosis and treatment of hereditary angioedema in Macedonia
5. *D. Moldovan, Cs. Todea:*
Hereditary angioedema: present and perspectives in Romania
6. *S. Cimbollek, T. González-Queved, M. Díaz Fernández:*
Experience and goals in Familial angioedema from the South of Spain

Saturday, 30 April – continued

7. *A. Blanch, O. Roche, T. Caballero, N. Sastre, D. Callejo, M. López-Trascasa:*
Patient registry and approach to the prevalence of HAE in Spain
8. *K. Obtulowicz, G. Porebski, B. Bilo, M Kapusta, P. Obtulowicz:*
Diagnostic and therapeutic problems in management of patients with HAE in Cracow/Poland
9. *M.M. Gompels, R.J. Lock, M. Abinum, C.A. Bethune, G. Davies, C. Grattan, AC. Fay, H.J. Longhurst, L Morrison, A. Price, M Price, D. Watters.:*
C1 inhibitor deficiency: UK Consensus Document.
Improving standards in the UK

15:45-16:00 Coffee break

16:00-16:50 Patients' Associations

Chairpersons: *A. Castaldo, M. Raguet*

1. *A. Menendez, I. Nagy, V. Penna, A. Price, M. Raguet, S. Smith, A. Castaldo, M. Cicardi:*
HAEI – International Patient Organization for C1 Inhibitor Deficiencies
2. *C. Picavet, L. Baas:*
Dutch patients and the patients association

**17:20 Intellectual experience,
...then a feast for all five senses**

Sunday, 1 May

08:30-10:15 Therapy

Chairpersons: *K. Bork, A. Agostoni*

1. *U. Huffer:*
Experiences with HAE Long-Term Medication: A Report from the German Patient Organization
2. *I. Martinez-Saguer, E. Aygören-Pürsün, E. Rusicke, S. Knaub, T. Klingebiel, W. Kreuz:*
Pharmacokinetics of Pasteurized C1 Inhibitor, (Berinert in 40 Patients with Hereditary Angioedema
3. *C. Symons:*
Plans for a UK Register of patients who self-infuse C1-INH concentrate at home
4. *K. Bork, G. Meng, P. Staubach:*
Treatment of abdominal pain attacks with pasteurized C1 inhibitor concentrate in patients with hereditary angioedema
5. *J.M. Muñoz, J. Jurado, T. Caballero, E. Vázquez, N. Prior, C. López-Serrano, V. Martorell:*
Oral surgery in patients with C1 inhibitor deficiency. Our results and protocol
6. *M. Bulnes, M.T. Caballero, M. Pedrosa, N. Prior, C. Cabañas, M.C. López-Serrano:*
Clinical evaluation of tolerance and viral safety of C1-inhibitor concentrate in patients with recurrent angioedema caused by hereditary or acquired C1-inhibitor deficiency
7. *W. Kreuz, I. Martinez Saguer, E. Aygören-Pürsün, E. Rusicke, T. Klingebiel:*
Individual replacement therapy (IRT) with a pasteurized C1-Inhibitor concentrate compared to prophylaxis with danazol in patients with Hereditary Angioedema (HAE) – a prospective study comparing quality of life parameters

Sunday, 1 May – continued

8. *A. Zanichelli, L.C. Zingale, B. Cicardi, L. Maggioni, E. Pappalardo, M. Cicardi:*
Use of C1 inhibitor concentrate for treatment of angioedema attacks in patients with C1 inhibitor deficiency. Survey of 1102 infusion in 503 patients

10:15-10:30 Coffee break

10:30-12:00 Results of new clinical trials

Chairpersons: *M. Cicardi, B Zuraw*

1. *S. Knaub:*
C1 Esterase Inhibitor, Human (Pasteurized) in Subjects with Hereditary Angioedema
2. *K. Bork, J. Frank, W. Kreuz, L. Dong, B. Rosenkranz, J. Knolle:*
Novel approach to treatment of hereditary angioedema with Icatibant, a bradykinin receptor antagonist
3. *B. Rosenkranz, L. Dong, J. Knolle:*
Pharmacokinetic and pharmacodynamic profile of Icatibant, a bradykinin B2 receptor antagonist
4. *J. Nuijens, R. Verdonk, M. van Doorn, R. Schoemaker, A. Cohen, E. Hack, G. Choi, M. Soeters, M. Levi, H. Farkas, L. Varga, J. László, G. Temesszentandrás, B. Bilo, G. Porebski and K. Obtulowicz:*
Clinical studies of Recombinant Human C1 Inhibitor in Subjects with Hereditary Angioedema, HAE
5. *M. Cicardi:*
DX-88 Clinical Experience Across EDEMA Trials by Localisation of HAE Attack

Sunday, 1 May – continued

6. *B. Zuraw:*
A Multicenter, Double-Blind, Placebo-Controlled Study of DX-88 in Hereditary Angioedema: Results of the EDEMA1 Study
7. *T. Craig:*
Multicenter, Open-Label, Repeat-Dosing Study of DX-88 in HAE: Interim Results of the EDEMA2 Study

12:00-13:00 Closing Remarks

13:00-14:30 Lunch

14:30- Departure

A B S T R A C T S

Course of two concomitant acute angioedema attacks in a HAE- patient treated with s.c. Icatibant

E Aygören-Pürsün, I Martinez Saguer, E Rusicke, T Klingebiel, W Kreuz
University Hospital Frankfurt, Germany

The inhibition of the Bradykinin B2-receptor by Icatibant (Jerini Ag, Berlin), a highly specific B2- receptor antagonist, is a new therapeutic concept for treatment of acute angioedema in C1-INH deficient patients. We report the course of two concomitant angioedema attacks in one patient, which were treated with 45 mg s.c. Icatibant.

A 31-year old male with severe hereditary angioedema due to C1-INH-deficiency type I presented with a severe acute abdominal attack, which was preceded by a concomitant mild angioedema of the foot. The patient was treated with a s.c.-injection of 45 mg Icatibant, 3:49 h:min after onset of abdominal symptoms.

Onset of symptom relief started 0:14 h:min after administration of Icatibant. Symptoms were markedly alleviated and the patient was completely free of symptoms 20 hours post-injection (p.i). The initially increased bradykinin serum level (91 fmol/ml, normal range 0.2-7.1) decreased to 19 and 27 fmol/ml (4h and 20h p.i.), whereas C1-INH activity and C4-level remained markedly below normal range. The drug was well tolerated, only a mild-moderate local reaction was documented. In the further course, 21:20 h:min after application of Icatibant, angioedema of both arms occurred, which required administration of 1500 U C1-INH concentrate in total.

Icatibant lead to rapid initial response of 2 concomitant mild/severe angioedema attacks in one patient. Still, in patients with severe HAE, further angioedema episodes may follow the administration of Icatibant. Antagonism of the BK B2 receptor with Icatibant represents a new therapeutic concept that may prove useful in the therapy of HAE.

The C4 serum level in Polish patients with hereditary angioedema

B Bilo, G Porebski, K Obtulowicz, M Kapusta, P Obtulowicz

Department of Clinical and Environmental Allergology, Jagiellonian University, Cracow, Poland

The measurement of C4 level is commonly used in diagnosis and follow-up of the C1 inhibitor deficiency. The aim of the study was to analyze C4 level in Polish patients with hereditary angioedema (HAE) at diagnosis and in therapy.

The measurements were done with nephelometry in 92 patients from 36 families (87 patients with HAE type I, 5 with HAE type II). In this group a total 202 measurements were performed (in 30 patients only one assay). The normal level of C4 for this assay according to the manufacturer is 0.1-0.4 g/l.

The mean C4 level measured for diagnostic purpose was 0.076 g/l [0.071 in 82 patients with previous symptoms, 0.11 in 10 previously asymptomatic patients]. In 21 (23%) newly diagnosed patients the level was above 0.1 g/l, in 7 (7.6%) - above 0.15 g/l. We did not observe a significant relationship between C4 level and frequency of symptoms, while C4 level was slightly but significantly higher in patients treated with androgens ($p < 0.01$). C4 level above 0.1 g/l was observed in 51 measurements (25%) in 31 patients (34%). Only 16 of these results occurred during androgen. Levels above 0.15 g/l were observed in 12 patients (13%) in 19 measurements (9%) (8 measurements during therapy). We observed a significant correlation between C4 level and C1 inhibitor level ($r = 0.57$, $p < 0.001$) and C1 activity ($r = 0.33$, $p < 0.001$) in HAE-I patients.

Even if C4 measurement is considered valuable as a screening test for HAE, normal (above 0.1 or even 0.15 g/l) levels are frequently observed in Polish patients with HAE. These false negative results only in part depend on treatment and their presence in untreated patients remains to be explained. Conclusion: A normal value of C4 serum level does not rule out HAE and further testing may be required.

Patient registry and approach to the prevalence of HAE in Spain

*A Blanch¹, O Roche¹, T Caballero², N Sastre³, D Callejo³,
M López-Trascasa¹*

¹Immunology, ²Allergy and ³Biostatistics Departments of Hospital Universitario “La Paz”, Madrid, Spain.

We developed a Spanish HAE patient registry to study the prevalence and the current situation of diagnosis and treatment of this disease in Spain.

Epidemiological data were obtained by direct contact with physicians who treat patients with HAE and with patients themselves. Diagnosis was assessed by measuring C1-Inh levels/function, and most families also underwent genetic studies.

We registered 444 patients (minimal prevalence 1.09:100000 inhabitants), many of whom are asymptomatic (never having symptoms) (61, 13.7%). Most symptomatic patients receive long-term prophylaxis treatment (62.9%) with attenuated androgens (80.9%) and antifibrinolytic agents (22.8%), alone or in combination, but no patients are receiving long-term prophylaxis with C1-Inh. There is a long delay in diagnosis (mean 13.1 years). Nine patients underwent a tracheotomy as a consequence of a laryngeal attack, and 30 families recalled a total of 38 relatives who died of HAE, which underlines the severity of the illness.

The detected minimal prevalence of HAE in Spain is 1.09:100,000 inhabitants. Because this is a rare disease and some patients may be misdiagnosed, this prevalence could be higher.

This work has been partially sponsored by ZLB Behring.

Novel approach to treatment of hereditary angioedema with Icatibant, a bradykinin receptor antagonist

K Bork¹, J Frank², W Kreuz³, L Dong⁴, B Rosenkranz⁴, J Knolle⁴

¹Department of Dermatology, University of Mainz, Germany, ²Department of Dermatology, RWTH Aachen, Germany, ³Center of Pediatrics, JWG University Hospital Frankfurt/M, Germany; ⁴Jerini AG, Berlin, Germany

Bradykinin (BK) most likely is a key mediator in acute attacks of hereditary angioedema (HAE) due to C1 inhibitor deficiency. BK plasma levels are increased during acute attacks and in C1 inhibitor knock-out mouse, Icatibant (HOE 140, JE 049), a highly specific bradykinin B2 receptor antagonist, reduced fluid extravasation and edema formation.

The efficacy and safety of Icatibant in acute HAE attacks have been assessed in this open-label study. Twenty (20) acute HAE attacks (10 cutaneous, 3 abdominal, 7 cutaneous and abdominal) in 15 patients with HAE were treated with Icatibant.

Treatment was started <10 hr after the beginning of an attack as follows: (n=4, each treatment group): a) 0.4mg/kg body weight (BW) over 2 hr i.v.; b) 0.4 mg/kg BW over 0.5 hr i.v.; c) 0.8 mg/kg BW over 0.5 hr i.v.; d) 30 mg s.c.; e) 45 mg s.c. Treated HAE attacks were evaluated by questionnaires and visual analogue scales. Treated attacks were compared with similar, untreated attacks reported previously by the same patients (10 to 600 attacks per patient). Treatment with Icatibant considerably shortened the time between beginning of the attack and resolution of symptoms. The duration of the treated attacks was shorter than that of previous attacks. Icatibant was well tolerated.

Icatibant may be of considerable therapeutic value for patients with hereditary angioedema due to its rapid onset of effect and the feasibility of self-administration by s.c. injection.

Treatment of abdominal pain attacks with pasteurized C1 inhibitor concentrate in patients with hereditary angioedema

K Bork, G Meng, P Staubach

Department of Dermatology, Johannes Gutenberg University, Mainz, Germany

Hereditary angioedema (HAE) due to C1 inhibitor deficiency clinically presents with recurrent and self-limiting edema of various organs. Abdominal edema attacks are associated with colicky, mostly severe pain and often combined with vomiting and diarrhea.

We studied the efficacy of a pasteurized C1 inhibitor concentrate (Berinert P, ZLB Behring, Germany) in HAE patients with abdominal attacks. Between 1976 and 2003, a total of 4,834 severe attacks in 75 patients were treated with 500 or 1,000 units of Berinert P. The quality and severity of pain, vomiting, diarrhea, and the course of the attacks were documented during personal interviews using standardized questionnaires and scores and compared with 14,721 severe and untreated abdominal attacks in the same patients who served as an intra-individual control group.

Relief of symptoms (pain and abdominal tension) was found to occur within 2 hours after infusion in 4,469 (92.6%) of the attacks and in 69 (92%) patients. The mean duration of the abdominal attacks was reduced from 93.8 ± 42.7 hours (untreated attacks) to 33.4 ± 22.1 hours (treated attacks). The mean maximal pain score of the untreated severe abdominal attacks was 9.6 ± 0.5 (10.0 would be absolutely unbearable pains) and was reduced to a mean pain score of 3.1 ± 2.0 in the treated attacks. Vomiting was documented in 13,200 untreated attacks of 71/75 patients and only in 291 treated attacks of 21/75 patients ($p < 0.0001$). Diarrhea was reported in 6,836 untreated attacks of 54/75 patients and in 531 treated attacks of 18/75 patients ($p < 0.0001$). There were no drug-related side effects.

The pasteurized C1 inhibitor concentrate Berinert P is highly effective and safe in treating severe abdominal attacks in patients with HAE suggesting a high impact on the quality of life of treated patients.

A HAE-III case associated with C1-INH cleavage without serpin-protease associations

L Bouillet¹, D Ponard², C Drouet²

¹Département de Médecine Interne, ²Laboratoire d'Immunologie, CHU Grenoble & Université Joseph Fourier, Grenoble - France

HAE-III diagnosis was established for Mes R born in 1972. Her sister presented edema attacks exacerbated with contraceptive pill. The patient presented the first attack at the age of 19, and she suffered monthly from edema, with 3 laryngeal and frequent abdominal attacks. Corticosteroids and antihistaminic were inefficient and contraceptive pill worsened the disease. C1-INH function was found 90% the reference value. Her first daughter was born in 1997 in the context of a pregnancy with severe attacks. In 1999 the treatment with tranexamic acid (1g x 3/day) was efficient. In 2001, a painfully second pregnancy was dramatic, with an *in utero* death nearly the date. There was no aetiology for this event. During the delivery, C1-INH function was decreased to 50% and found associated with important cleavage of C1-INH protein (immunoblot). After delivery, C1-INH function raised to normal except during attacks where C1-INH function strongly decreased. The third pregnancy in 2004 was very painful, with weakly attacks. No treatment was administrated during the three months, but tranexamic acid was from the second term. A laryngeal edema attack required C1-INH concentrates. Finally she was hospitalised during the last month and delivery was prepared with C1-INH concentrates. Both baby and mother were well. During development of pregnancy C1-INH function progressively decreased to nearly 50% at delivery, with extensive serpin cleavage without serpin-protease associations. Normal C1-INH function was observed after delivery and disease improved. In a HAE-III patient, this observation reports for the first time that attacks are more frequent during pregnancy and are associated with strong decreased C1-INH function in parallel with its proteolysis.

This work was supported by a grant from the thematic action 5 of the 2002 Programme Hospitalier de Recherche Clinique.

Clinical evaluation of tolerance and viral safety of C1-inhibitor concentrate in patients with recurrent angioedema caused by hereditary or acquired C1- Inhibitor deficiency

M Bulnes, MT Caballero, M Pedrosa, N Prior, C Cabañas, MC López-Serrano

Department of Allergology, University Hospital, La Paz, Madrid, Spain

The therapy of choice of acute attacks of angioedema caused by hereditary or acquired C1-INH deficiency in Spain is C1-INH concentrate, available since 1970's.

We evaluated tolerance and safety of C1-INH concentrates, before and after the introduction of virucidal methods, in 64 patients (62 with HEA), regularly seen in our service since 1980. The patients were divided into two groups: 1) 35 treated patients; 2) 29 untreated patients. Blood borne viral infections were evaluated by serological assays (ELISA to HIV, HBV, HCV, Parvo Virus).

One patient had seroconversion to HCV after use of non-virus-inactivated concentrate. None had any seroconversion after the introduction of virucidal methods. No significant side effects have been recorded.

Beriner[®], pasteurized and lyophilized C1-INH concentrate, in use in Spain, is well tolerated and safe.

Effect of mutations within the coding region of C1-INH gene on protein function in families with HAE

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C1 esterase inhibitor (C1-INH) is a serine protease inhibitor (serpin). Serpins are a superfamily of proteins that fold into a conserved structure and utilize a unique suicide substrate-like inhibitory mechanism. The native structure has a metastable conformation that is required for serpin inhibitory activity. When the target protease recognises and cleaves the scissile bond in the reactive site loop (RSL), this flexible loop, initially exposed to the solvent, starts to insert into β -sheet A and bring the covalently bound protease with it. Upon complete loop insertion the proteinase is translocated by over 70 Å, and its active site distorted. The energy needed to complete the inhibitory process comes from the higher stability of the locked-in conformation compared with the native-like conformation.

The picture of C1-INH looks like that of other serpins. However, C1-INH tertiary structure is not experimentally known and systematic functional and structural studies on this inhibitor are not abundant. We are now screening for mutations in C1-INH gene a case list of 471 HAE patients belonging to 179 independent families. At present we have identified 132 different mutations in C1-INH gene. In order to gain insights onto the structure-function relationship of C1-INH, we selected those mutations affecting the mechanism of loop insertion (delThr280, Tyr308Asn, Arg378Cys, Leu427Pro/Arg) to be expressed in *Pichia pastoris* and functionally characterized.

The requirement for a metastable conformation in the native/active state renders serpins extremely sensitive to point mutations resulting in conformational instability and polymerization. The deleterious effects of serpins polymerization are well known (Alzheimer, Parkinson diseases, etc), while effects on minor conformational changes are just now revealing to act as signals for a range of physiological responses. The structural and functional properties of these targeted mutants could contribute to elucidate the biological activities of serpins in general, providing useful insights into the pathogenesis of conformational diseases and could also identify genotype-phenotype correlates in HAE to be exploited for new therapeutic strategies.

DX-88 clinical experience across EDEMA Trials by location of HAE attack

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DX-88, a novel recombinant protein, is a highly specific, potent kallikrein inhibitor that has shown significant therapeutic benefit in patients experiencing the acute attacks associated with hereditary angioedema (HAE). Presented here is a summary of the clinical benefits of DX-88 at the various anatomic sites evaluated in the DX-88 phase II EDEMA trials in HAE.

EDEMA0 is a European-based, open-label, multicenter phase II dose-escalation study; EDEMA1 is a randomized, double-blind, placebo-controlled, phase II dose-escalation study; and EDEMA2 is an open-label phase II study that is evaluating the safety and efficacy of repeat administration of DX-88 in patients experiencing recurrent attacks secondary to HAE.

In general, clinical response was equally good at all anatomic sites. In EDEMA0, all 9 patients showed onset of symptom relief within 4 hours of dosing: 4/4 patients with facial attacks, 2/2 with abdominal attacks, 1/1 with a combined peripheral/abdominal attack, and 2/2 with peripheral attacks. The median time to the onset of symptom relief for the DX-88–treated patients across all attack sites was 81.8 min (range: 5–120 min). In EDEMA1, a significantly greater proportion of the DX-88–treated patients, as compared with those in the placebo group, experienced onset of symptom relief within 4 hours of initiation of dosing: 29/40 (72.5%) DX-88 patients *vs* only 2/8 (25.0%) in the placebo group ($P=0.017$). Results by anatomic site were 13/19 (68.4%) *vs* 1/3 (33.3%) patients for abdominal attacks ($P=0.53$), 3/3 (100%) *vs* 1/1 (100%) for laryngeal attacks, and 13/18 (72.2%) *vs* 0/4 (0%) for peripheral attacks ($P=0.007$). In EDEMA2, among 61 attacks evaluated in the interim analysis, there was no substantial difference in clinical response rates between attack types.

These results demonstrate that DX-88 can alleviate the symptoms of HAE across various attack sites. Additional clinical experience at other attack sites, as well as selected HAE case studies, will also be discussed.

Experience and goals in Familial Angioedema from the South of Spain

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Since 1989 our Allergy Unit in the south of Spain has become reference center for patients diagnosed of Hereditary Angioedema (HAE).

Over the last 15 years 15 families have been screened of which 76 members are affected by deficiency of C1 inhibitor (C1 INH), three of them only functional. 69 of them are symptomatic. One family, where up to four generations have been studied is a model of biochemical alteration without clinical symptoms. Systematically, blood and urine analysis, coagulation, hormones including testosterone and SHBG and complement serum levels are evaluated. Also, yearly abdominal ultrasound are realized.

Long term treatment is mainly achieved with low doses of attenuated hormones (Danazol 50-150mg) and tranexamic acid. For acute attacks patients dispose of detailed info cards and C1 INH plasma concentrate at home also used for short term prophylactic purposes. Good control has been achieved with little side effects. Analytical alterations, decreased libido, breast atrophy, amenorrhea and psychological alterations have been observed in some patients. Nine patients have undergone genetic study. Two *de novo* mutations, two large deletions and previously unreported heterogeneous mutations affecting exon 3 and 5 have been detected so far. Particularities like 2 pair of twins, 2 pregnancies without incidences, congenital hypothyroidism, administration of kallikrein inhibitor and elevation of CA 125 during an abdominal attack will be discussed.

A regional patient meeting is about to be organized in order to join efforts, raise awareness, optimize treatment and provide platform for further research in this rare disease.

A multicenter, open-label, repeat-dosing study of DX-88 in HAE: interim results of the EDEMA2 Study

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DX-88 is a highly specific, potent plasma kallikrein inhibitor that has been shown in clinical trials to be a promising agent for the effective and safe treatment of the acute attacks associated with hereditary angioedema (HAE). Reported here is the interim analysis of EDEMA2, one of the 3 phase II trials in HAE.

EDEMA2 is an ongoing trial at 21 US and Canadian centers that is evaluating the safety and efficacy of DX-88 when administered repeatedly, as a 10-minute intravenous infusion, to patients experiencing separate attacks of HAE. The interim analysis was performed on data derived from the treatment of 61 attacks in 34 patients treated at multiple dose levels (5, 10, and 20 mg/m²) as of November 2004. Various HAE attack sites were evaluated, including peripheral, abdominal, and laryngeal (48%, 41%, and 11% of patients, respectively). Of the 34 patients, 15 were treated with DX-88 multiple times (8 separate attacks treated in 1 patient).

DX-88 provided beginning of resolution of HAE symptoms within 4 hours of initial dosing at all attack sites. Rates of clinical response, as defined by the beginning of improvement of HAE symptoms within 4 hours of dosing, were similar at the different dose levels: 92% response at 5 mg/m², n=24; 91% response at 10 mg/m², n=22; and 100% response at 20 mg/m², n=15. The median time to clinical response was 35 minutes across all dose levels. However, differences in the durability of response were observed between dose levels. Specifically, there was a substantially lower rate of rebound attacks for the combined results from the 10 and 20 mg/m² dose levels (3%), as compared with the 5 mg/m² dose level (25%). No drug-related serious adverse events were reported.

Data from this interim analysis of EDEMA2 further support the conclusion that DX-88 may provide substantial therapeutic benefit at all attack sites in patients experiencing acute HAE attacks, with no apparent decrease in efficacy upon administration of multiple doses. The results continue to indicate that DX-88 has a good safety profile and is well tolerated.

Anti-inflammatory activities of C1 inhibitor that are independent of protease inhibition

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Over the past two years, at least two potential new anti-inflammatory activities of C1 inhibitor (C1INH) have been described. In the first, both active C1INH and reactive center cleaved, inactive C1INH were shown to protect mice from lethal Gram-negative endotoxemia. Both forms of C1INH blocked the LPS-binding protein-dependent binding of *Salmonella typhimurium* LPS to a murine macrophage cell line (RAW 264.7), and inhibited LPS-induced TNF- α mRNA expression by these cells and by human blood cells. The interaction of C1INH with LPS was demonstrated by ELISA and by non-denaturing PAGE, but deletion of the amino terminal non-serpin domain abrogated this binding. Removal of *N*-linked carbohydrate also resulted in diminished ability to protect mice from LPS-induced septic shock, and did not suppress LPS binding to cells, or LPS-induced TNF- α mRNA expression. *N*-deglycosylated C1INH bound to LPS very poorly. Removal of *O*-linked carbohydrate had no effect on any of these activities. In recent studies, we have characterized the binding site for LPS within the amino terminal domain and have demonstrated that treatment with C1INH decreases mortality in the cecal ligation and puncture model in mice.

In the second potential anti-inflammatory activity, C1INH was shown to react with monoclonal antibodies to the sialyl Lewis^x tetrasaccharide, which is an important ligand for selectin adhesion molecules. Plasma C1INH binds to P and E-selectins, as demonstrated by FACS and immunoprecipitation experiments. Using tissue culture models, C1INH suppressed endothelial-leukocyte adhesion and transmigration in a dose-dependent manner. Using an *in vitro* flow chamber model, both native C1INH and reactive-center cleaved C1INH suppressed selectin-mediated leukocyte adhesion to both E and P selectins, while *N*-deglycosylated C1INH loses such activities. C1INH also suppressed TNF- α -induced leukocyte rolling and sterile thioglycollate-induced peritonitis in mice. The data support the hypothesis that C1INH may play a direct role in leukocyte-endothelial cell adhesion. Current studies are directed toward defining the relative roles of inhibitory activity and inhibition of leukocyte transmigration in C1INH-mediated suppression of inflammation.

Mission is possible

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A huge gap is known exist worldwide between the wealth of information on HAE and the current level of health care services rendered to afflicted patients. HAE is an uncommon disorder and therefore, centralizing the management and follow-up of patients is a prerequisite to efficient care. This is substantiated by examples from several countries, where high-quality patient care has been developed through many years of diligent effort and complex co-operation with the self-help organizations of patients. Efficient HAE centers can assist regions where patients are unable to access appropriate care owing to the insufficient knowledge of medical professionals; lack of diagnostic facilities; or because adequate therapy is unaffordable. Deliberate distribution of experience and support for establishing the necessary infrastructure can accelerate the foundation of HAE centers in these countries, and this shall provide the means for the rapid and efficient delivery of services for large patient populations. The Regional HAE Network was established in 2004. A three-year development program has been drafted focusing on education, the introduction of state-of-the-art diagnostic and therapeutic methods, as well as the organization of a nationwide HAE registry and patient organization. Individual chapters of this project have already been launched in, Bulgaria, Macedonia Romania and Ukraine – this report describes the interim results of our mission.

The support for this project – including the distribution of financial grants from pharma companies such as Pharming, Jerini, ZLB Behring, and Dyax – is integrated by the Hungarian HAE Foundation.

The effect of sexual hormone alterations on the frequency of oedematous attacks in patients with hereditary angioneurotic edema

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Changes in the body's hormonal equilibrium may alter the frequency of angioedema attacks in patients with hereditary angioneurotic edema. We assessed the relations between the angioedema attacks and puberty, menstruation, anticoncipient pill taking, pregnancy, delivery and menopause. We also studied the possible impact of an embryo with hereditary angioneurotic edema on the frequency of attacks during pregnancy.

We involved 53 women in our retrospective analysis. We used questionnaire surveys and made extensive gynecological examinations. We pointed out that the frequency of the attacks increased in 34% of the patients in puberty, at the time of menstruation in 58% of the patients and in 63% of the contraceptive pill users. In 36% of the women the number decreased in postmenopause. In case the pregnancy affected the disease, the embryo with hereditary angioneurotic edema increased the number of attacks during pregnancy.

Our analysis shows, that conditions with sexual hormon alterations have an effect on the number of edematous attacks, therefore, the patients with hereditary angioneurotic edema need more attention both in nursing and therapy, when they are in a lifeperiod of hormonal change. Our results offer the oppurtunity of a better prediction of edematous attacks, thus the oppurtunity of better therapy and life quality.

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Mutation analysis of patients with hereditary angioedema in Germany

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In 1999, we set up a genetic testing program of the *C1INH* gene for HAE patients and until now we have screened 210 families comprising 354 members clinically suspicious for the disease. Point mutations and small rearrangements were identified by direct sequencing of all 8 exons and adjacent intronic regions. Large deletions were initially detected by Southern blotting and are now screened for by a quantitative DHPLC assay.

In 30 families with 59 members, we found mutations that have been described before, especially mutations at amino acid 444 which are responsible for HAE type II. In 85 families with 144 members, we found 72 novel mutations. The mutation spectrum of our sample is composed of 28/72 (38,8%) missense mutations, 9/72 (12,5%) nonsense mutations, 25/72 (34,7%) small deletions, insertions and duplications, 10/72 (13,8%) splice site mutations. Nine families with 22 patients had large *C1INH* deletions.

The C1 inhibitor gene mutation database (HAEdb <http://hae.biomembrane.hu/>) reports on 128 mutations in the *C1INH* gene. The spectrum of mutation types in our data set corresponds well to the published data.

Routine molecular genetic analysis can identify the causative mutations in most HAE families. Early diagnosis of mutation carriers prior to clinical manifestation is essential for prevention and treatment of acute and life threatening edema.

Recombinant expression of C1-Inhibitor mutants confirms causality of missense mutations for hereditary angioedema

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In order to study the functional effect of C1INH mutations we have expressed the C1-INH protein in human cells. The cDNA of *C1-INH* was cloned into the expression vector pCEP4 and transfected into HEK 293 cells. Recombinant C1INH protein was recovered from the culture medium and its activity was measured by a chromogenic assay. The presence of C1INH antigen was checked by Western blotting and hybridisation with an anti-C1INH-antibody. Mock transfected HEK cells showed neither activity nor antigen, thus ruling out any endogenous C1INH production. Wild type C1INH showed an activity and antigen comparable to diluted human plasma. Ten missense mutations observed in HAE patients (M-22V, A-21V, A134D, S233T, L374P, L376P, L376Q, R378C, R444G, V458G) and one known polymorphism (V458M) were introduced by site directed mutagenesis and expressed in HEK 293 cells. While all recombinant protein variants representing missense mutations resulted in a significant decrease of C1INH activity, thus proving their causality for HAE, the polymorphism did not significantly alter C1INH function.

In conclusion, recombinant expression of C1INH protein variants is a useful tool to analyse the causality of missense mutations and to characterize the role of critical amino acid residues within the C1INH protein.

C1 Inhibitor improves outcome in neonatal cardiopulmonary bypass

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Patients with Hereditary Angioedema lack functional product of one of the two C1 INH genes. They have attacks of capillary transudation and angioedema formation, often in response to trauma. We suggest that we create the functional equivalent of hereditary angioedema when we prepare newborns for cardiopulmonary bypass surgery. Newborn children have their blood diluted 50% with salt solutions immediately before undergoing the trauma of bypass surgery. C1 INH levels fall to ½ to of adult levels and complement is activated. Capillary permeability and swelling, pulmonary dysfunction, and cardiac dysfunction often occur. We studied whether C1INH will reduce the inflammatory response to CPB in neonates.

47 neonatal pigs (2.5 ± 0.4 kg) were assigned to a control group, (N=19) or C1INH group (N=28) that received intravenous C1INH (500, 1000, or 1500 IU, Aventis Behring) prior to CPB. All animals underwent CPB via sternotomy at $100 \text{ ml/kg}^{-1} \text{ min}^{-1}$ for 90 minutes at 37°C . Pulmonary and cardiovascular variables were measured before CPB and at 5, 30, and 60 min. post-CPB and analyzed using repeated measures ANOVA.

C1INH concentration in the plasma post-CPB increased linearly with increasing dose. Pulmonary function 60 minutes after CPB improved with increasing C1INH dose, as measured by higher dynamic lung compliance ($P < 0.001$) and lower A-a gradient ($P = 0.014$). Total body weight gain was less in the high dose group (mean 0.24 ± 0.10 kg vs. 0.38 ± 0.09 kg, $P = 0.002$). Cardiac systolic function at 60 minutes after CPB was better in the high dose group, as measured by $\text{dP/dt}_{\text{max}}$ ($P = 0.016$), but the time constant of diastolic isovolumic LV relaxation was slower in the high dose group (60 ± 6 ms vs. 31 ± 3 ms, $P = 0.003$).

C1INH supplementation results in improved pulmonary and cardiovascular function 60 minutes after CPB in a neonatal pig model. The basis of the positive effect on systolic function and the negative effect on diastolic function is yet unknown. C1INH may reduce morbidity associated with CPB in the neonate.

C1 Inhibitor deficiency: UK Consensus Document – Improving standards in the UK

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Most health care professionals in the UK are not familiar with C1 inhibitor deficiency. Wide variation in management strategies has led to major differences in quality of life outcomes for patients. The UK C1 inhibitor consensus document was commissioned by the Primary Immunodeficiency Association (PIA) and was written by a panel of UK physicians, specialist nurses, scientists and patients, all with extensive experience of managing C1 inhibitor deficiency. Advice was based on published evidence and where this was not available, expert opinion.

Major treatment recommendations include:

- the opportunity for each patient to keep C1 inhibitor concentrate at home.
- a major expansion of access to home therapy (self infusion) with C1 inhibitor.
- C1 inhibitor for management of all attacks which interfere with normal activities, including abdominal attacks.
- use of plasma for acute attacks is strongly discouraged.

We recommend that all patients should have access to a centre with a special interest in C1 inhibitor deficiency, via a local specialist if necessary. We stress the importance of the nurse specialist, the dentist, the emergency physician and the well-informed patient.

We hope that this document will serve as a guide to physicians, patients and funding bodies, and will enable most people with C1 inhibitor deficiency to live a normal and productive life.

Experiences with HAE long-term medication: a report from the German Patient Organisation

U Huffer

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The German HAE Patient Organisation was founded in 1997. Today 280 members have been registered, we know about 540 families affected by HAE in Germany.

In the past, as in many other countries, Danazol (Androgen derivate) was the only treatment for prophylaxis for HAE-patients in Germany. A pasteurised C1-Inhibitor concentrate (Berinert[®]P, C1-INH) has been available for treatment of acute attacks in Germany since 1985. Danazol is no longer registered in Germany. New medications for treatment of acute attacks in HAE are under development.

I myself was severely affected by HAE attacks for all my life. I received Danazol for over 20 years, and most of that time it was a real benefit to me. But Danazol suddenly did not work any longer to prevent my attacks. As a consequence, the medical centre at the University Hospital in Frankfurt switched my therapy to individual replacement therapy. I had to learn how to practice self-infusion with C1-INH. Unfortunately the side effects I developed under Danazol did not disappear completely. Like me many other German HAE patients have reported similar experiences. Other patients are still using Danazol and are happy with the treatment while another group of patients eagerly expects new treatment options. However, for most of our members, it is C1-INH, which allows them to work and to go to school. Thus a “normal” life is possible. From my point of view, I may say that although we all suffer from the same disease, an adequate treatment for each individual patient can only be defined on the basis of a patients individual symptoms and personal situation.

In any case, we should keep in mind the risk / benefit ratio of any long term therapy, especially in case of those HAE patients who suffer from frequent and severe attacks.

C1 esterase inhibitor, human (pasteurized) in subjects with hereditary angioedema

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Beriner[®] P is a highly purified pasteurized, human C1-Inhibitor concentrate (C1-INH), which has been on the market for 20 years. C1-INH is regarded as the treatment of choice for patients who suffer from acute attacks of hereditary angioedema (HAE) as it provides the natural missing protein to the patient. As the natural enzyme it has a positive impact on all pathways involved in the pathophysiology of HAE. Ample clinical and post marketing data with Beriner[®] P confirm that the preparation is safe and effective and provides rapid relief of symptoms associated with all kinds of edema, including the very painful abdominal attacks and the life threatening laryngeal edema. So far, no rebound of an attack has been described in the literature after treatment with Beriner[®] P and the virus safety of the product is well documented. Pharmacokinetic data with Beriner[®] P demonstrate that with a half-life of around 40 hours the preparation is suitable to effectively carry out prophylaxis every 2-5 days in certain patients with a high frequency of HAE attacks. The short $t_{T_{max}}$ of around 1 hour is consistent with the rapid onset of clinical efficacy for C1-INH in patients suffering from acute HAE attacks.

Beriner[®] P provides the natural protein to patients suffering from hereditary deficiency of C1-Inhibitor and is therefore regarded as the treatment of choice. It has been shown to be safe and effective in the treatment of acute HAE attacks and its pharmacokinetic properties allow to effectively carrying out prophylactic treatment in patients with frequent HAE attacks.

Individual replacement therapy (IRT) with a pasteurized C1-Inhibitor concentrate compared to prophylaxis with danazol in patients with hereditary angioedema (HAE) – a prospective study comparing quality of life parameters

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Attenuated androgens like danazol are the current treatment of choice for long-term prophylaxis in patients with hereditary angioedema (HAE). Unfortunately, this group of drugs bears the risk of severe side effects (e.g. depression, weight gain, hirsutism, transaminase elevations, liver adenoma and carcinoma, headaches, hypertension, menstrual abnormalities).

We therefore explored the option to administer a pasteurized, plasma-derived C1-Inhibitor concentrate (pC1-INH, Berinert P) for individual replacement therapy (IRT) in patients with HAE in which danazol induced severe side effects, was not effective or was contraindicated (e.g. children, pregnant women).

Prospective, observational, intra-individual cross over study, comparing efficacy, safety and quality of life of danazol (prophylaxis) vs. pC1-INH (IRT) in 23 patients suffering from severe HAE.

All patients, who received pC1-INH (IRT) showed significantly decreased mean annual attack frequency and were free of life threatening attacks or danazol related adverse events. All quality of life parameters like family/home responsibility, occupation, social activities, general condition and condition during attacks improved significantly; $p < 0.001$. The mean stay in hospital in patients receiving danazol prophylaxis was 15.3 ± 22.9 days compared to 0 days in HAE patients receiving pC1-INH (IRT). During the entire observation period with pC1-INH in these patients there was no viral transmission.

Compared to danazol, pC1-INH (IRT) in patients with severe HAE significantly reduces the frequency of HAE attacks and significantly improves all quality of life parameters.

Do the mannan-binding lectin pathway of complement activation and some infections contribute to the pathogenesis of hereditary angioedema?

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Hereditary angioedema (HAE) is severe disease, characterized by the defect in the C1-inhibitor of complement which is a key factor in its pathogenesis. The mannan-binding lectin (MBL) pathway of complement activation has been studied, since two proteins of this pathway are hampered by C1-inhibitor. MBL concentration, MBL pathway activity, and the other possible factors in pathogenesis – infections with *Helicobacter pylori*, HBV, and HCV, were investigated in HAE patients.

The study was performed in 66 patients: 19 children and 47 adults. The control group consisted of 91 healthy children and adults. No significant differences were found between median values of MBL in patients (children and adults) and control group (MBL concentration: 573 vs 725 ng/ml; MBL activity: 339 vs 373 mU). However, median functional index (FI) was significantly higher in HAE patients than in controls ($p < 0.05$). The highest values were in those patients whose disease symptoms appeared at age < 10 years (median functional index = 0.729). The frequency of anti-*Helicobacter pylori* IgG antibodies in children (42%) differed from that in children from general population (27%). No serological markers of ongoing infections with HBV nor HCV were found. However, positive anti-HBc results were noted: 1/19 children (5.26%) and 7/42 adults (16.7%; ~3 times higher than general population). All anti-HBc(+) samples were HBV-DNA negative.

Functional index value of the MBL pathway may be connected with the onset of HAE. Both *Helicobacter pylori* (in children) and HBV (in adults) were demonstrated as previous or possibly accompanying infections.

Pharmacokinetics of pasteurized C1-Inhibitor, (Berinert \hat{a}) in 40 patients with hereditary angioedema

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Hereditary deficiency in C1 inhibitor (C1-INH) function frequently results in potentially life-threatening attacks of hereditary angioedema (HAE). A highly purified and pasteurized C1-INH concentrate is available to effectively treat angioedema attacks in patients with hereditary C1-INH deficiencies, but relatively little is known about its pharmacokinetic properties. **Objective:** Pharmacokinetics and *in vivo* recovery (IVR) of C1-INH concentrate (Berinert[®] P) were evaluated in patients with HAE who receive this preparation either as individual replacement therapy (IRT, regular, immediate treatment of first HAE symptoms in patients with frequent and severe attacks) or as on-demand treatment. **Methods:** Forty subjects (15 under IRT, 25 under on-demand treatment) with HAE received intravenous injections of C1-INH concentrate (542-1,617 U) in an attack-free interval in a prospective, open, uncontrolled, single-center study. Blood was sampled for determination of C1-INH activity for up to 72 hours after dosing. Pharmacokinetic parameters were calculated using a single-compartment model and IVR was determined using standard methods. **Results:** The mean (\pm SD) time to maximum plasma concentration (T_{max}) for C1-INH administered in patients under IRT was 1.3 ± 2.1 hours, the area under the time versus plasma concentration curve (AUC) was 20.5 ± 19.1 hour U/mL, the elimination half-life ($t_{1/2}$) was 33.3 ± 19.8 hours, mean residence time (MRT) was 48.0 ± 28.5 hours, total body clearance (Cl) was 1.1 ± 0.6 mL/kg/hour, and volume of distribution at steady state (V_{ss}) was 39.5 ± 9.9 mL/kg. The respective values for patients treated on demand were 2.9 ± 6.5 hours, 20.0 ± 14.5 hour U/mL, 43.9 ± 22.4 hours, 63.4 ± 32.3 hours, 1.2 ± 1.0 mL/kg hour, and 51.4 ± 10.9 mL/kg. The mean IVRs for IRT and on-demand treatment were $108.2 \pm 48.3\%$ and $85.8 \pm 28.3\%$, respectively. Children tended to have slightly lower half-life and a slightly higher V_{ss} compared to adults. **Conclusions:** C1-INH concentrate has a short T_{max} and a long $t_{1/2}$ and MRT. This is consistent with the rapid onset of clinical efficacy for C1-INH concentrate in subjects suffering from HAE attacks and the ability to effectively carry out IRT with injections administered every 2-5 days.

HAEI - International Patient Organization for C1 Inhibitor Deficiencies

A Menendez, I Nagy, V Penna, A Price, M Raguet, S Smith, A Castaldo, M Cicardi

HAE International (HAEI) is the umbrella organization for the world's HAE patient Associations, and is now a legally recognized charitable entity registered in Nancy, France. The need for an international approach to HAE patient advocacy, and the idea to establish HAEI, was spawned during a fall 2002 meeting of HAE Patient Association leaders organized by Professor Marco Cicardi in Palermo, Italy.

HAEI's mission is to promote co-operation, co-ordination and information sharing between HAE Patient Associations, and facilitate the availability of effective HAE diagnosis and management throughout the world. To achieve its mission HAEI has set ambitious objectives that include:

- Developing and supporting of National Member Organizations throughout the world.
- Maintaining a website to serve as an informative online resource for the HAE global community.
- Organizing international workshops to foster interaction among the entire HAE community (medical, scientific and patients)
- Promoting scientific and clinical research on new treatments for C1-INH deficiencies.

A distinguished group physicians and researchers has agreed to serve on HAEI's Medical Advisory Panel because they recognize the value of forming an international alliance to reach HAE patients and health care providers throughout the globe.

This Workshop is also serving as HAEI's organizational kick-off meeting.

Hereditary angioedema: present and perspectives in Romania

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Romania is far behind many nations in caring for patients with hereditary angioedema (HAE). The awareness of HAE among general practitioners and even specialists is very low. No HAE centre is available at this moment, and no register of HAE patients exists. None of the patients referred to my allergist colleagues with suspicion of HAE had a C1 inh antigenic protein and C1 inh functional assay screening made in Romania. Previous efforts to register C1 inhibitor purified extracts from plasma were unsuccessful.

It is our intention to start to set up a HAE centre in Tîrgu Mure^o. Our project includes a broad cooperation with general practitioners, specialists in allergology, dermatology, critical care, surgery and pediatric. A national HAE network is under construction, and powerpoint presentations, leaflets, articles in papers from excellent reviews are in work. We are going to start a lobby to support this category of patients with potential serious outcome at governmental level. International support would be of paramount importance.

Characterisation of a mutation of the *C1NH* gene at splice sequence and associated with HAE-I

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HAE-I diagnosis was established in two independent kindreds suffering from angioedema upon low C1-INH antigenic and functional levels. We next identified the mutation g640A>G in intron 2 of the *C1NH* gene, at position +3 relative to exon 2 (c.111+3A>G). No other mutation/rearrangement were detected in the *C1NH* gene of both families.

To analyse transcript maturation and establish the responsibility of the intron 2 mutation on the C1-INH deficiency, monocyte monolayers were prepared from the index patient. Supernatants were harvested and RNA were prepared. Unexpectedly C1-INH secretion was found quite comparable to control monocytes. RNA species were examined by RT-PCR using 3 sets of primers encompassing sequences of exons 1 to -3, -1 to -4 and -1 to -5. Wild-type transcript represented the major species and was associated with minor bands of Mr compatible to deletions of the only exon 2, the only exon 3 and both exons 2 and 3. A minor exon 3 skipping was also observed in control monocytes. C1-INH production was assayed in parallel in monocyte culture from HAE-I patient with exon 4 deletion, with strongly impaired C1-INH secretion as previously reported.

The low levels of circulating C1-INH observed in these kindreds are assumed to be mainly dependent on liver secretion. These data suggest a discrepancy of splicing efficiency between monocytes and hepatocytes. Efficient although limited recognition of the modified donor splice-site sequence by monocytes could explain subsequent minor exon 2 skipping. Besides the hepatocyte expression is more affected, with the mutation associated with complete loss of the splicing site.

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Metallopeptidase activity and kinin metabolism in hereditary angioedema (HAE): effect of androgen prophylaxis

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Human aminopeptidase P (APP) exists in both cytosolic (hcAPP) and membrane-bound (hmAPP) forms, the latter being most likely responsible for plasma activity. The metallopeptidases play an important role in the catabolism of kinins, mostly for des-Arg⁹-bradykinin (des-Arg⁹-BK). We reported an impaired degradation of this endogenous active metabolite of bradykinin (BK) associated with a low plasma APP activity in cases of hypertensive patients suffering from angioedema (AE) while treated with ACEi (Molinario et al, *J Pharmacol Exp Ther* 303: 232). Pathophysiology of HAE is presently attributed only to a quantitative/qualitative defect of C1-INH with an increased liberation of BK. We hypothesize the accumulation of vasoactive kinins in HAE is due not only to a C1-INH defect but also to a low activity of plasma APP. The purposes are: 1- to define the values of plasma APP activity and kinin metabolism in a group of HAE-I and -II treated or not with danazol, 2- to compare these metabolic profiles with these of a reference population.

163 HAE-I and -II patients and 119 healthy individuals were investigated for the plasma APP activity and the metabolism of kinins as described (Adam *et al. Lancet* 2002; 359: 2088).

Results: 1- When compared with the reference values, mean plasma APP activity was significantly decreased in the samples from some HAE patients without androgen prophylaxis, 2- mean plasma APP activity was strongly increased upon androgen prophylaxis, 3- the values of APP activity could be related with kinetic parameters which characterize the degradation of plasma BK but mainly des-Arg⁹-BK.

These data open new area of research on the pathophysiology of HAE and on the mechanism of the prophylaxis with androgens.

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Oral surgery in patients with C1 inhibitor deficiency – our results and protocol

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Oral surgery in patients with C1 inhibitor deficiency may cause laryngeal edema and high risk of death. In the past, without treatment, global death after dental surgery was 30-40%. Last decade, although different treatments are available, four cases of death have been described in the literature.

We reviewed the cases of oral surgery in patients with C1 inhibitor deficiency in our hospital during the last four years to evaluate complications, laryngeal oedema and comfort of the patients

A number of 16 dental surgical proceedings have been performed in 11 patients. Two of them were performed in Operating Room and the other 14 in consulting room in dental chair as usual in normal patients. The problems treated were 2 dental cysts, 8 wisdom teeth, 28 exodontias and root removing and drainage of one dental abscess. All were performed under local anesthesia. The two proceedings carried out in Operating Room spent 24 hours in hospital, the rest were treated with no admission. Preparation was done with iv. C1 inhibitor concentrate (Berinert®) in all cases plus increased dose of attenuated androgens in four cases. No complications have been observed, neither facial or laryngeal edema. Only one case of dental abscess post-exodontia that was treated with drainage with preparation with C1 inhibitor concentrate under local anesthesia. The follow up was done by the allergist and the maxillofacial surgeon and the patient had a contact telephone for consultation and a dose of Berinert® for complications before the admission in Emergency Room of our hospital in case of laryngeal edema.

We advocate the use of local anesthesia for these treatments, with no admission regimen as normal patients. C1 inhibitor concentrate may be used as the only preparation. Multidisciplinary team and HAE knowledge of the surgeons facilitate the correct dental treatment of these patients. The higher number of dental proceedings may be carried out in the same episode.

Psychiatric disorders in Hungarian patients with hereditary angioneurotic edema

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Psychiatric disorders in patients with hereditary angioneurotic edema had never been screened before, although they can provoke edematous attacks. We started a study in 2004 in the Hungarian HAE Center to screen depression, anxiety, neurotic disorders and the quality of life.

We used self-administered questionnaires: Shortened Beck Depression Inventory Scale (BDI), Spielberger State-Trait Anxiety Inventory (STAI-T), Juhász Neurotic Disorders Scale (JNEUR) and the Illness Intrusiveness Ratings Scale (IIRS). Our questionnaire contained questions about the patients' social and family life and about the HAE Center too.

Questionnaires were sent to all 105 Hungarian HAE patients, 80 of them was returned shortly after that. After estimation of 73 questionnaires, (43 female, 30 male; age: 5-75 years, mean: 32.47 years) we determined that the prevalence of these disorders are higher in our patients than in the normal Hungarian population or in outpatients with allergic diseases too. According to BDI 30, 1% of the patients scored above the normal level (> 10) and 17.8% had clinically significant depressive symptomatology (> 19). 26 patients (35.6%) scored above the normal level, 20 (27.3%) among them included into the high anxiety group (> 48) by the STAI-T.

We found serious psychiatric disorders in the case of 22 persons (30.13%). The incidence of depression, anxiety and neurotic disorders is significantly higher ($p < 0.05$) in the patients who often suffered from submucosal (laryngeal or abdominal) attacks.

Over 80% of our patients mentioned, that the control examinations, the multilingual info-card, the continuous phone-duty plays an important role in their life.

This study has proved the importance of screening and treatment of psychiatric disorders and the follow up of the patients. Decreasing the rate of the psychiatric disorders could improve the life of patients.

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Clinical Studies of Recombinant Human C1 Inhibitor in Subjects with Hereditary Angioedema, HAE

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Recombinant human C1INH (rhC1INH; purity > 99.98%) was isolated from the milk of transgenic rabbits for replacement therapy in subjects with HAE, who have a deficiency of functional C1INH in plasma. A Phase I study in asymptomatic subjects with HAE demonstrated that intravenous administration of rhC1INH at 100 U/kg provides functional C1INH in plasma at levels exceeding 0.4 U/mL for about 9 hours and that rhC1INH displayed biological activity through increasing C4 in plasma.

No clinically significant adverse events, changes in vital signs, safety laboratory parameters or clinically significant immune responses have so far been observed in ongoing open-label Phase II studies of the safety and effects of rhC1INH at 100 U/kg in severe acute HAE attacks. In all cases of abdominal (5), facial (2), urogenital (1) and peripheral (2) attacks, both subjects and physicians evaluated treatment with rhC1INH as favorable compared to previously untreated attacks. Time to the beginning of relief was between 15 min and 4 hours, whereas time to minimal symptoms varied between 1 to 8 hours for the abdominal attacks, and usually was within 24 hours for the attacks with involvement of subcutaneous tissue. None of the patients experienced a relapse of the initial attack.

The positive open-label trial results were reason to initiate randomized controlled trials to unequivocally demonstrate the efficacy and extend observations of the safety and tolerability of rhC1INH in symptomatic subjects with HAE.

Diagnostic and therapeutic problems in management of patients with HAE registered in Cracow/Poland.

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Hereditary Angioedema (HAE) in Poland seems to be underdiagnosed illness. In population about 40 million people 150 patients have been diagnosed. Aim of the study was to characterize the group of HAE patients diagnosed and treated in Cracow and to analyze the related clinical issues.

Currently (January 2005) Cracow registry includes 104 HAE patients from 44 families diagnosed and treated in our department. Diagnosis was based on the clinical history and examination during attack of angioedema as well as laboratory estimation of C1 and C4 serum concentration (nephelometric method) and concentration and the activity of C1 inhibitor and CH50 (enzymatic kinetic method). Type I HAE was confirmed in 99 patients (61 women, 38 men, in the age 6-34) and type II in 5 persons. At diagnosis mean laboratory values were: for the type I: C1inh – 0.09g/l, fC1inh – 19.3%, C4 – 0.07g/l, CH50 – 67.26% and for type II: C1inh – 0.64g/l, fC1inh – 31.1%, C4 – 0.15 g/l, CH50 – 59.4%. The most often symptoms were recurrent extremital oedema and abdominal symptoms, next the face, larynx and genitals swelling. In 10% of patients we detected time-dependent variation in C4 serum level, that sometimes reached normal values, not always during the androgen treatment. It seems that normal C4 serum level do not exclude the possibility of HAE. There were some cases when we observed the decrease of symptoms severity after the therapy of the inflammatory foci. 12 patients were treated with C1 inhibitor (50 occasion, Berinert P) because of life-threatening attacks but there is limited access to C1 inhibitor therapy in Poland. 15 patients were treated with Danazol for over 2 years. The treatment was usually effective according to symptoms but we observed normalisation in C4 and C1 inhibitor levels only in 4 patients. There are in observation also 30 persons from 10 families with typical HAE symptoms but normal C4 and C1 inhibitor level and C1 inhibitor activity, and 2 brothers with typical symptoms but laboratory confirmation only in one of them.

Since HAE is a rare disease it frequently remains undiagnosed and mistreated. Not all patients may be correctly classified because of the lack of adequate testing methods.

Identification of variables causing different clinical expression of hereditary angioedema

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Hereditary angioedema has a highly variable phenotype, but the reasons for such variability remain undisclosed. Identifying mutations and their molecular effect may be relevant for genotype/phenotypes as well as for structure/function correlates.

Our experimental approach is based on identification of mutations responsible for HAE by screening our case list of 471 HAE patients (179 independent families) characterized for clinical phenotype. At present screening has been completed in 132 unrelated patients: 112 type I, 19 II and 1 with an intermediate phenotype. We found 96 different mutations in 132 patients and none in 8. They consist in: 35 missense, 22 frameshift, 12 large deletions/duplications, 11 nonsense, 5 splicing defects, 3 insertion/deletion of 1–2 codons, 2 affecting the 1^oMet of signal peptide, 2 possible splicing defect, 1 missense/splicing defect, 1 gene deletion, 1 promoter point mutation and 1 synonymous mutation.

Another approach is based on association of FactorXII and ACE polymorphisms with different HAE phenotypes. Patients were divided in 3 groups, “severe”, “moderate” and “mild” based on the clinical phenotype. Ninety-eight patients and 50 controls were checked for Factor XII (C/T) and 93 patients and 50 for ACE (Ins/Del) polymorphisms. The C/C polymorphism in FXII is related to high level of protein transcription. We found an increment from “severe” (53%) to “moderate” (63%) and “mild” (74%) classes suggesting an inverse correlation between this polymorphism and disease severity.

The Del polymorphism in the ACE gene is related to higher plasma levels of protein. Our results showed that this polymorphism had a similar distribution between the group “mild” and controls (D/D 47%, I/D 42% and I/I 11%) while in the group “severe” it was less frequent (D/D 23%, I/D 67%, I/I 4%) than in controls. These data suggest that polymorphisms in the proteins involved in HAE symptoms pathogenesis may influence the clinical phenotype. More data are needed to validate these preliminary results.

Usefulness of abdominal ultrasound in the follow-up of patients with hereditary C1-inhibitor deficiency

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The aim of this study was to assess the usefulness of abdominal US in the follow-up of patients with hereditary C1-inhibitor deficiency in diagnosing acute attacks as well as in the follow-up of possible side effects of long-term prophylaxis with attenuated androgens.

61 patients with HAE regularly followed in our service were included whether they were symptomatic or not, and received androgen long-term prophylaxis or not. We evaluated the ultrasonographic findings in the assessments carried out routinely or in the moment of abdominal acute attack.

57 out of 61 patients had ever any symptom due to HAE (abdominal location in 77.19%). Abdominal US was performed in 47, with no significant findings in 32 of them. In 10 cases the US was performed during acute attack. Ascites and wall swallowing was found in 7 of 10 cases. 32 out of 61 patients were or had been under androgen prophylaxis and in 29 abdominal US assessments were carried out. 3 cases of angiomas, 5 of steatosis, 1 of portal hypertension, hepatic cysts and hepatomegaly respectively were found.

Abdominal ultrasonography has been proved useful as an early tool for diagnosing side effects of therapy, as well as for confirming diagnosis in case of abdominal acute attack.

Successful IVIg treatment of angioedema attacks in a patient with acquired C1INH deficiency and IgG deficiency

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We report here a female patient aged 75 affected with IgG deficiency and acquired C1-Esterase Inhibitor Deficiency successfully treated by means of high dose intravenous immunoglobulins (IVIg). The patient referred to our group because of repeated attacks of edema of the face, trunk, airways or abdominal viscera occurring spontaneously and frequently (1-2 times per week). Symptoms started at the age of 68 and consistently worsened during last months. Attacks did not ameliorate after corticosteroids or antihistamines administration. Cutaneous attacks were not pitting nor pruritic and usually lasted several hours. Abdominal attacks were often accompanied by diarrhea. The patient was affected also with repeated infections of the upper airways. Familiar history was negative for angioedema. Main laboratory finding were very low IgG and either functional or quantitative very low C1INH. C4 and CH50 were also very low and no antibodies against C1INH were demonstrated. DHPLC analysis did not find any mutation in the C1INH gene thus confirming the diagnosis of acquired C1INH deficiency. Lymphocyte subpopulations were normal. Treatment with IVIg was started because of the very low levels of IgG. Soon after the initiation of this treatment angioedema symptoms almost completely disappeared. Very mild symptoms were only sometimes present soon before each successive IVIg infusion. Clinical and laboratory findings during the six months of treatment with IVIg will be reported and discussed.

Germinal mosaicism and HAE

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We report here the case of two babies aged 5 and 2 affected with HAE in the absence of any positive familiar history. The patients came to our group because of repeated attacks of edema of the face, trunk and extremities occurring spontaneously; for one of them also laryngeal edema was reported. Main laboratory findings were very low either functional nor quantitative C1INH and low C4 in both babies, and low CH50 in one baby. Parents complement profile did not show any abnormality. DNA paternity was performed and resulted in a confirmation of legal parent. DHPLC analysis of C1INH gene found a mutation in both babies but not in their parents (and in other relatives). DNA sequence molecular studies of C1INH gene were therefore undertook in the two babies and, by the way, in their parents. We found in the babies the same nonsense mutation 531C>G (STOP codon Y177X → protein without 302 aa). Such mutation (nor others) wasn't found in the two parents. Therefore the possibility of a germinal mosaicism was examined. Linkage analysis was performed and the same aptotype was found in both babies. We found by this test the maternal germinal origin of the mutation that was confirmed by the consequent molecular studies of other maternal tissues (urin, sputum, skin) where mutations were absent.

Detailed aspects and future perspectives will be further discussed. This is the first report of germinal mosaicism in HAE.

C1-inhibitor therapy is effective in capillary leak syndrome due to chemotherapy – case report

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Capillary leak syndrome (CLS) is an unusual condition characterized by peridic leakage of plasma proteins through the capillary wall, leading to hypoalbuminaemia, hypovolaemia, hemoconcentration and shock. The pathogenesis is unknown and mortality high. CLS may complicate sepsis, may be induced by therapy with IL-2, following open heart surgery or a bone marrow transplantation.

20 years old woman with acute myeloid leukemia type M3 high risk (AML M3) was subjected to allogenic peripheral blood stem cell transplantation (PBSCT). The patient received a conditioning regimen consisted of Treosulfan in daily dose 12 g/m² for 3 days (on 6, 5, 4 day before transplantation). Reduced intensity regimen was chosen because of high probability of liver toxicity of Busulfan after Myelotarg [anti-CD33 with anthracycline] treatment in relapse. Cyclosporine (CSP) at dose of 3 mg/kg was administered as prophylaxis of Graft versus Host Disease. Early toxicity of chemotherapy resulted in CLS. The clinical manifestations of CLS: retention of fluids, resistance for furosemid: peritonitis, pericarditis, pleuritis and increasing body mass occurred 2 days after PBSCT. The patient was treated with haemodialysis and methylprednisolon at dose 2 mg/kg. On 22 day after PBSCT patient received C1 inhibitor at dose 500 U, subsequently the same dose was repeated after 11 days.

The effect of C1-Inhibitor already after the first infusion was estimated as favourable, because the clinical state of patient stabilized, her body weight normalized, pericarditis, peritonitis and pleuritis were reduced. This beneficial effect of C1-inhibitor therapy indicates that this drug even in a low dose is a promising approach for the management of patients with severe complication of bone marrow transplantation.

Dutch patients and the patient association

Ch Picavet, L Baas

Dutch HAE-QE patient association

The Dutch patient association has initiated a patient survey in order to assess patient experiences, both within and outside of medical settings. It also examines what patients expect of their association and how they evaluate the association's activities. The aim of this study was to increase the knowledge about the implications of HAE and QE (Quinke's Edema) for patients.

Two short questionnaires were developed for HAE and QE patients. They contained questions about medical history, symptoms, treatment, side-effects, and quality of life. Furthermore, questions were asked about the patient organization's services. The questionnaires were sent to all members of the association. 78 questionnaires were returned, which was a response rate of 63%.

The well-known long time before diagnosis became apparent in our (small) study. Average time between first symptoms and diagnosis is almost 11 years. Most people still have occasional swellings despite medicine use. The frequency and intensity of attacks vary between patients. For QE patients no standard treatment is available. They have more attacks and are more often troubled by facial swellings. About half of the respondents think the disease has some impact on their daily lives. Implications are reported in the domains of social relationships (especially for QE patients), and occupation or study. Also, some people found out it's harder to get health or life insurance.

Although many people think their disease has little or no impact on their daily lives, for some others the impact is huge. Some people suffer more or less continuously from attacks and are heavily limited in their social lives. Almost 40% think life is less worthwhile because of their disease. Implications for the patient organization and our services are discussed.

Health-related quality of life in adults with hereditary angioedema: development of a disease specific questionnaire in Spain (phase I)

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The health-related quality of life (HRQOL) in Hereditary Angioedema (HAE) due to C1 inhibitor Deficiency is undocumented. Multiple factors in assessing the HRQOL requires valid and reliable instruments. Although there are available generic questionnaires, such as SF-36, a disease-specific instrument is preferable as is more likely to be responsive than a generic measure to clinical change.

The aim of this study was to develop an specific questionnaire for measuring the functional (physical, social, emotional and occupational) impairments in the daily lives of HAE patients.

In this first phase we filled semi-structured interviews with a group of HAE patients elder than 18 years old in Madrid and Seville, as well as with experts in the disease of these two cities. A qualitative content analysis was made, grouping answers in categories, that were transformed into items to compose the disease specific questionnaire. A standardised form was developed to carry out an evaluation with respect to comprehensibility, adequacy and relevance to HAE by a group of experts and patients. General observations and suggestions were also taken into account to revise and rephrase the items, concluding the final preliminary version.

In a second phase validation of the questionnaire will follow up. The application of a generic HRQOL questionnaire (SF-36) will also be assessed.

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Identification of gene mutations of one Chinese Type II HAE family

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Objective: To identify the mutation of C1 inhibitor (*CIINH*) gene in a Chinese HAE type II family.

Methods: Using polymerase chain reaction (PCR) to amplify the exon eight of the C1 inhibitor gene and followed with gene sequencing. Sequencing results were compared with the normal sequences in GenBank to find the mutations.

Results: Codon CGC was converted to TGC at position 18059, which corresponding to an Arg → Cys replacement at position 444.

Conclusions: We found a common point mutation within the reactive centre, which had been reported as the second most frequent mutation in type II HAE.

A case of hereditary angioedema with abdominal pain and ascites

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Hereditary angioedema (HAE) is a rare autosomal genetic disorder that is characterized by submucous and subcutaneous angioedema usually affecting the face, extremities, upper airway and abdomen. Rare cases with abdominal pain and ascites with HAE episodes have been reported so far. We report a patient which developed abdominal pain during acute episode which involved her face. Ultrasound demonstrated transitory ascites in this case.

Characterization of splicing mutations and identification of a possible exonic splicing enhancer in the *CINH*

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We have screened the entire *CINH* for mutations in a large series of 87 Spanish HAE families and we have characterized at the mRNA level several defects affecting the splicing process.

Mutation analysis was performed by single strand conformation polymorphism (SSCP), sequencing, Southern blotting and quantitative multiplex PCR. The mutations affecting the splicing were characterized by reverse transcriptase-PCR.

We found 10 different mutations, affecting either canonical splicing sequences of exons 2, 3, 4, 5 and 7, or the last base of exon 3. The mutation c.551-2delA (g.4349delA) produces exon 4 skipping, c.686-3C>G (g.8322C>G) and c.889+2T>C (g.8530T>C) exon 5 skipping, and c.1249+2delT (g.14252delT) exon 7 skipping. In addition, our mRNA studies revealed for the first time two splicing defects related to the presence of exonic mutations. The mutation c.884T>G (g.8523T>G; Leu273Arg) produces three mRNA species: one with exon 5 deleted and two normally spliced, corresponding to the wild type and to the one which carries the mutation c.884T>G. The mutation c.882C>G (g.8521C>G; Tyr272X) only produces the wild type mRNA and an aberrantly spliced transcript that skips exon 5.

In conclusion, we characterized the effect of 4 mutations affecting the canonical splice sites of exons 4, 5 and 7, and we identified a possible exonic splicing enhancer (ESE) motif within the 3' end of exon 5.

Pharmacokinetic and pharmacodynamic profile of Icatibant, a bradykinin B2 receptor antagonist

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Icatibant, a potent and specific bradykinin (BK) B2 receptor antagonist, has shown efficacy in the treatment of acute HAE attacks (see separate presentation). Bradykinin antagonism and s.c. bioavailability have been demonstrated in two Phase I studies as described below.

In 18 healthy males, safety, pharmacokinetics and pharmacodynamic profile have been assessed. Part I (3 panels of 4 subjects) compared single 1h and 4h infusions (0.005 to 3.2 mg/kg) in an ascending, double-blind, placebo-controlled design. Part II tested a 24h (0.15 mg/kg/day) vs. repeated 1h infusions (0.5 mg/kg q8h) in a double-blind cross-over design. Icatibant concentrations were assessed using LC-MS/MS, and the pharmacodynamic response by the degree of inhibition of the effect of repeated i.v. BK challenges using an individually titrated dose causing a 10-15 mmHg blood pressure drop. Competitive, dose and concentration dependent inhibition of BK-induced tachycardia (plethysmography) and facial flush (laser-Doppler flowmetry) was obtained. Icatibant showed a rapid distribution, an elimination half-life of ~2h to 4h and linear pharmacokinetics over the dose range tested. It was well tolerated up to a dose of 1.6 mg/kg.

In a second crossover study, 24 subjects received Icatibant (0.4 mg/kg) either s.c. or via an i.v. infusion over a 30 min period with a washout phase of 7 days. Absorption after s.c. injection was reproducible, complete and rapid with a bioavailability of 92%, a T_{max} of 0.6 h, and a CV of 23% for AUC.

Icatibant was safe and well tolerated in the dose range intended for the treatment of HAE attacks.

In conclusion, Icatibant is a specific, competitive inhibitor of BK in man; its rapid and complete bioavailability allows s.c. administration for the treatment of HAE attacks.

Improvement of clinical course in hereditary angioedema following modification of life style

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It is general clinical experience that the manifestation of Hereditary Angioedema (HAE) shows broad interindividual variability as well as considerable intraindividual fluctuation over time. A variety of external influential factors are known to have impact on manifestation of HAE in terms of attack frequency.

We report on 6 patients with sole on demand C1-Inhibitor therapy (1 male, 5 females; median age 30,5 years, range 19-33) who showed a marked reduction of angioedema attacks during the observation time (median 4 years, range: 2-6). In this period a median decrease of attack frequency from 5 attacks/month (range: 1-12) to 1 attack/month (range: 0,3-3) was documented. The reduction of attack frequency was associated with modification of life style or nutritional habits: Two patients started with psychotherapy, two patients used nutritional additives, in one patient no special reason could be observed. Severe swelling attacks of face and larynx as well as abdominal attacks were successfully treated with C1-Inhibitor-concentrate (500- 1500 IU Berinert) on demand. The reduction of attack frequency was accompanied by a decrease in C1-Inhibitor-concentrate consumption in all patients.

The clinical courses of our patients with Hereditary Angioedema show considerable changes over time after life style modification.

Diagnosis and treatment of hereditary angioedema in Macedonia

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OBJECTIVES: To present patients with hereditary angioedema diagnosed and treated at Clinical Center Skopje.

METHODS: Retrospective review of the patients referred for evaluation and treatment of recurrent angioedema.

RESULTS: A total of 3 families with hereditary angioedema have been found in Macedonia. The frequency of symptoms was highly variable from patient to patient. Two twin sisters were diagnosed in their early childhood period at University Children Hospital, and four are diagnosed at adulthood at University Clinic of Dermatovenerology. All had C1 inhibitor deficiency and low levels of C4 diagnosed at Institute of immunology and human genetics, Medical Faculty Skopje. The patients were treated and are followed up at Clinic of Dermatovenerology. Acute attacks were treated with fresh frozen plasma and Trasyolol. Some of the patients are receiving long-term prophylaxis with attenuated androgens.

CONCLUSIONS: We are aware that the present situation in Macedonia regarding registration, diagnosis, treatment and follow up of patients with HAE should improve in the future. Introducing the HAE center will enable us to foster knowledge of this disorder in Macedonia and to advance care of patients with this disorder.

HAE patients healthcare and management in Bulgaria – history, present and future

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The past and present status of the clinical, immunological and molecular diagnostics as well as treatment and healthcare of hereditary angioedema (HAE) in Bulgaria is presented. The entity has been first reported in Bulgaria in 1973. There are more than 120 HAE patients, both family and sporadic cases. There is no systematic organization of the diagnostics and treatment, performed mainly at the casual basis. Adequate diagnostic tests have been carried out mainly abroad on limited cohort of patients in the course of research studies. Patients are addressed to different kind of specialists without *a priori* training. The specific treatment is not covered by the health insurance system or any other official financial source.

Future plans and perspectives are outlined on the base of first HAE workshop in Bulgaria, held in March 2005.

Plans for a UK Register of patients who self-infuse C1INH concentrate at home

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Members of the Royal College of Nursing Immunology and Allergy Nurses Group (RCN I&ANG) have been training patients and their partners with Hereditary and Acquired Angioedema to infuse C1 esterase inhibitor concentrate at Home.

At our Spring 2004 meeting it was decided that a Register of such patients should be kept by one of the Home Therapy training centres so that any adverse reactions could be documented and the safety of this means of delivery could be evaluated. A group of Nurses have held similar Registers for those patients who infuse IVIg or SCIg at Home for more than 15 years and it has been a useful audit tool. Results published from this documentation show very few problems for those patients who have self-infused IVIg or SCIg.

Using these documents as a template, a similar pro forma for C1INH Home Therapy has been drawn up. It is intended that the form will be completed by the patient and the reporting Nurse, and all the information will be held centrally. Any adverse events can then be collated and the material used to assess the efficacy of this means of treatment.

This documentation will be presented to the RCN I&ANG in March 2005. There will be a discussion of the Data Protection issues surrounding the electronic holding of patient details at the I&ANG meeting.

After approval by the Members, we hope to be able to put the Register into effect later this year.

Adverse effects of long-term danazol prophylaxis on the lipid profiles of patients with hereditary angioedema: a possible risk factor for atherosclerosis

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In the treatment of hereditary angioedema (HAE) danazol -an attenuated androgen- is commonly used for long-term prophylaxis. Our objective in the present study was to investigate the adverse effects of danazol on the serum lipid and lipoprotein profiles of the patients and to determine whether the treatment is associated with increased risk of atherosclerosis or not.

Detailed lipid profiles of 37 adult HAE patients receiving long-term danazol prophylaxis, 27 HAE controls (who have never received continuous danazol therapy, HAE controls), and 66 age and sex matched healthy controls were determined. Concentrations of total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, apolipoprotein A-I, apolipoprotein B-100 and lipoprotein(a) concentrations were compared between the danazol treated HAE patients and the two control groups.

Serum concentrations of high-density lipoprotein ($p=0.0002$, $p<0.0001$) and apolipoprotein A-I ($p=0.0015$, $p<0.0001$) were significantly lower, whereas low-density lipoprotein ($p=0.0129$, $p=0.0127$) and apolipoprotein B-100 ($p=0.0456$, $p=0.0013$) were higher in the danazol treated patients compared to both groups, respectively. We found no significant difference in the total cholesterol, triglyceride or in lipoprotein(a) levels. Our patients who received danazol had higher risks for abnormally low high-density lipoprotein (OR: 11.6 (2.7-49.7), $p=0.0010$) and high low-density lipoprotein (OR: 4.4 (1.2-16.0), $p=0.0264$) concentrations.

We demonstrated that long-term danazol prophylaxis significantly modifies lipid and lipoprotein profiles of patients with HAE and it should be considered as a risk factor for atherosclerosis in these patients. Consequently, monitoring of high-density lipoprotein and low-density lipoprotein levels at regular intervals is recommended during the follow-up.

This work was supported by grants from the Ministry of Welfare (ETT 194/2003) and the EC Contract (QLG1-CT-2002-01359).

Normal Complement C4 does not exclude hereditary angioedema (HAE)

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Assay of C4 and C1 esterase inhibitor antigen (C1inhA) and function (C1IF) is essential for the diagnosis of HAE and useful for disease monitoring. Many UK laboratories only perform C1inh analysis in patients with low C4 levels, although in our experience a normal C4 may occasionally be compatible with the diagnosis of HAE. Functional analysis of C1inh activity is problematic, with several kits in use; at our centre, a functional ELISA (Technoclone Diagnostics, Dorking, United Kingdom) has proved to be a robust technique.

We evaluated the results of tests for C4, C1inhA and C1inhF in the St Bartholomew's HAE cohort. Patients with chronic idiopathic urticaria with C4 and C1inh studies performed as part of their routine investigation served as a comparison group.

Several patients with untreated HAE (confirmed by C1inh analysis and complotyping) had C4 values within the normal range (0.14g/L). Using a normal range of $150\text{-}450\text{mg/L}$, C1inhA was 100% specific and sensitive for the diagnosis of HAEI. The Manufacturers' range for C1IF (<60% considered abnormal) appeared to be too low; by restriction of curve analysis, a lower limit of normal of 84% provides 80% sensitivity and 100% specificity for the diagnosis of HAEI and II using C1inhF alone.

We conclude that a normal C4 level does not exclude HAE, and that C1inh studies should be performed if there is a high index of clinical suspicion. In-house normal ranges are often preferable to Manufacturers', which should be treated with caution.

HAEdb: a novel interactive, locus-specific mutation database of the C1 inhibitor gene

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Hereditary angioneurotic edema (HAE) is an autosomal dominant disorder characterized by episodic local subcutaneous and submucosal edema and is caused by the deficiency of the activated C1 esterase inhibitor protein (C1-INH). Published C1-INH mutations are represented in large universal databases (OMIM, HGMD), but these databases update their data rather infrequently, they are not interactive and do not allow searches according to different criteria. The HAEdb, C1-INH gene mutation database (<http://hae.biomembrane.hu>) was created to contribute to the following expectations: (i) help the comprehensive collection of information on genetic alterations of the C1-INH gene, (ii) create a database where data can be searched and compared according to several flexible criteria, (iii) provide additional help in new mutation identification. The database is maintained in the server of the Department of Molecular Cell Biology at the National Institute of Hematology and Immunology (Budapest, Hungary). The web site uses MySQL, an open-source, enterprise level, multithreaded, relational database management system. The user-friendly graphical interface was written in the PHP web programming language. The web site consists of two main parts, the freely browseable search function, and the password-protected data deposition function. Mutations of the C1-INH gene are divided in two parts: “gross” mutations involving DNA fragments >1 kb and, “micro” mutations encompassing all non-“gross” mutations. Several attributes (e.g. affected exon, molecular consequence, family history etc.) are collected for each mutation in a standardized form. This database may facilitate future comprehensive analyses of C1-INH mutations and also provide regular help for molecular diagnostic testing of HAE-patients.

Enzyme-linked immunosorbent assay (ELISA) of functional and total C1-esterase inhibitor (C1-INH)

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The principal laboratory tests for establishing the diagnosis of hereditary or acquired angioedema are measurements of the serum or plasma levels of functional C1-INH activity and total C1-INH immunoreactivity, supplemented with determination of C4 and C1q. The most commonly used analyses of functional and total C1-INH differ in assay principle and format, the former measuring the inhibition of C1s activity by means of a chromogenic substrate, the latter measuring total C1-INH protein by a variety of immunochemical methods. Functional and total C1-INH assays use separate calibrators related to mean normal activity levels or protein levels respectively. Existing tests of functional C1-INH activity are reported to give a proportion of either falsely low readings in the enzymatic method or falsely high readings in an alternative immunochemical method. We have developed a sandwich ELISA in which it is possible to measure functional and total C1-INH and relate these to a single calibrator of purified normal C1-INH. This enables the functional and total C1-INH levels in a given patient to be directly compared. The coating antibody captures C1-INH in the sample whether or not it is complexed with C1s. This is an advantage over capture of C1s, as it avoids interference from excess added C1s, which is washed away. For the functional assay, diluted samples and calibrator are pre-incubated with an excess of activated C1s. C1s complexed with coat-bound C1-INH is measured with a labeled C1s antibody, which enables the functional activity of the C1-INH to be measured down to very low levels and, if necessary, compared with total C1-INH measured in unreacted samples with a labeled C1-INH antibody. Validation of these new analyses is in progress to assess their performance against the current methods.

Lectin pathway activity of complement is depressed in hereditary angioedema (HAE)

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The role of the lectin pathway (LP) in the pathogenesis of the angioedema symptoms in HAE has not been studied. LP is initiated by mannose-binding lectin (MBL). MBL genotypes A/O and O/O are associated with low serum levels of MBL and low activity of the LP pathway. Our aim was to evaluate the role of the three complement pathways in HAE.

The activities of the classical pathway (CP), LP and alternative pathway (AP) were measured by functional ELISA-based kits (Wielisa) in the samples of 96 patients with HAE and 30 healthy controls. In addition were determined MBL genotypes by PCR.

Low LP activity was found in patients compared to controls (31.1 vs 120.3; $p < 0.008$) in homozygous carriers of the normal MBL genotype (A/A), but not in carriers of variant genotypes (A/O, O/O). Activity of CP correlated with LP in patients, but no correlation with AP was observed. The activities of both CP and LP correlated with CH50, C1q, C4, antigenic/functional C1 inhibitor, while the activity of AP correlated with C3, CRP and age. No differences were found in the activities of the three pathways between HAE type I and II. The activity of LP was lower ($p = 0.019$) in the patients with onset of symptoms before 10-year-age compared to those with over 10-year-age. We found higher CP ($p = 0.04$) and AP ($p = 0.009$) activities in patients with severe angioedema symptoms compared to those with less severe symptoms. Both activities of CP ($p = 0.009$) and LP ($p = 0.007$) were higher in patients on long-term danazol treatment compared to patients without danazol treatment, but there was no difference in the activity of AP. Higher activities of AP were associated with laryngeal attacks ($p = 0.016$) and mechanical trauma as triggering factor ($p = 0.001$).

Based on our data parallel measurement of activity of the three pathways of complement has major clinical importance in HAE. However analysis of data involving more patients would be necessary to confirm these results.

This work was supported by ETT 522/2003 and Wieslab, Lund, Sweden

Haematologic adverse reactions during danazol treatment

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In our case report we introduce a rare adverse reaction during danazol treatment.

K.T. was a 15 years old boy suffering from HAE, when we started danazol therapy in November of 2001. Previously despite of the tranexamic acid prophylaxis, he suffered from frequent oedematous attacks. At the next visit (6 months later, in May of 2002) he was symptomless. After 11 months danazol therapy his behaviour became aggressive, he suffered from headache, when we detected erythrocytosis. Despite of the decreased doses of danazol, erythrocytosis and thrombocytopenia were detectable in November of 2002, too. Bone marrow examination didn't show clonal disease. Danazol treatment was stopped in December of 2002.

Without anabolic steroid therapy the HAE symptoms developed more and more frequently. At about 11 months later, in November of 2003 we started danazol therapy as a new model. K.T. received 100 mg danazol therapy three following days every week. Two weeks later he was aggressive again. Finally 50 mg danazol with 2 mg methylprednisolon were administered with good experiences, without adverse reactions.

We've never seen any similar case in our practice.

This work was supported by the ETT 194/2003 grant.

N. M., the first hereditary angioedema (HAE) patient from Ukraine

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The female patient, N. M. was born in 1952. Her first angioedema swellings of the extremities manifested at the age of 3. Between the age of 14 and 27 years she was suffering from angioedematous swellings of the extremities, gastrointestinal tract and larynx, usually 2-3 times per month. Conservative therapy with antihistamines and corticosteroids had been ineffective.

The patient was observed at the Immunology Scientific and Research Institute, Moscow from 1980 till 1985, where the diagnosis of HAE had been determined. She was treated with fresh frozen plasma and aminocaproic acid. Since the age of 27 she underwent tracheostomy three times, and she suffered from laryngeal chondroperichondritis and acute venous thrombosis of the subclavian, underarm and humeral veins after the removal of a subclavian catheter. Her symptoms occurred more and more frequently – the disease showed progression.

Due to the difficulties experienced in Ukraine, her family became to look for an opportunity to treat the patient abroad and to get C1-inhibitor (C1-INH) concentrate (Berinert P500). From 2002 the Hungarian HAE Center helped the patient with medical consultation and with C1-INH concentrate (by the kind support of Aventis/ZLB Behring). The Hungarian HAE Center asked the patient – N. M. – to contact us, Ukrainian allergologists, who are interested in establishing a local HAE Centre in Ukraine.

In this case, the patient was the one who found her doctors.

Start of the program to study hereditary angioedema (HAE) in Ukraine

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Before the year 2004 we did not have any information about HAE: we had only one case; but we had no complement laboratory, no treatment options, no HAE Center, thus we had no knowledge about HAE. As the population of Ukraine is about 48 million, the estimated number of patients with HAE could be as high as 800-4000. Our goal was to improve the quality of the diagnosis and treatment of HAE in Ukraine.

Step 1: the first patient N. M. asked the Hungarian HAE Center for help in 2002. She was invited to the 3rd C1-INH Deficiency Workshop, in Budapest to meet other patients and medical. Her blood was tested and she received C1-INH concentrate (Berinert P500). The patient got in touch with the Hungarian Center and received medical consultation after on.

Step 2: The Hungarian Center decided to establish the Regional HAE Network to help other countries, which do not have experience of managing HAE patients.

Step 3: The Hungarian Center asked the N. M. – our first patient - to contact Ukrainian colleagues, who are interested in this problem. In December 2004 Hungarian colleagues participated at the Ukrainian Immunodeficiency Conference, presenting two lectures on the clinical picture, diagnosis and treatment of HAE and about the Hungarian Center. We decided to work together.

Step 4: To explore the number of affected patients, we asked Ukrainian colleagues - allergologists and immunologists - about their patients suspected with HAE. We collected their samples and filled out questionnaires on epidemiology data.

Step 5: Hungarian Center invited us to take part in a training course on HAE in Budapest. We studied the laboratory methods, received reagents and our collected samples were analyzed.

Our plans are: to write articles in professional journals, to conduct lectures for colleagues and to hold workshops about HAE in Ukraine. The main aim is to establish the national HAE register by collecting patients and their blood samples.

Use of C1 inhibitor concentrate for treatment of angioedema attacks in patients with C1 inhibitor deficiency – Survey of 1102 infusions in 503 patients

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Replacement therapy with C1-INH concentrate is the treatment of choice for severe acute attacks of HAE and AAE in Europe.

We report the need and efficacy of C1-INH concentrate in 477 patients with HAE and 25 with AAE observed for: 30-25 years 50 patients, 24-20 in 44, 19-15 in 48, 14-10 in 96, 9-5 105, <5 in 159. Efficacy was evaluated as the time to beginning of resolution of symptoms. Side effects were recorded. C1-INH concentrate was used in 149 of 477 HAE patients (31%) for a total of 1013 infusions (mean infusion/patient 6.8, range 1-84) and by 8 of 25 AAE patients (32%) with a total of 89 infusions (mean infusion/patient 11.1, range 1-27). The dose ranged from 500U to 2000U for HAE, 1000U-12000U for AAE.

In 431 laryngeal attacks time to beginning of resolution was between 30 and 60 minutes in 424 episodes (98%). In 475 abdominal attacks time to beginning of resolution was within one hour in 469 episodes (99 %). Among 21 episodes of edema of the oral mucosa time to beginning of resolution was within one hour in 20 (90 %). In 39 facial attacks the time to beginning of resolution was within 60 min in 26 (67%). In 45 peripheral attacks the time to resolution was within one hour in 23 episodes (51 %). In 5 patients with AAE (54 infusions) beginning of resolution was always within one hour; in 3 patients the response became progressively slow 3 requiring higher doses of C1-INH concentrate. Our data indicate that treatment with C1-INH concentrate is highly effective in angioedema of the laryngeal or abdominal mucosa; its effectiveness is reduced when the skin is involved and particularly in peripheral attacks. Patients with AAE may become resistant to the treatment. Safety is generally good.

A multicenter, double-blind, placebo-controlled study of DX-88 in Hereditary Angioedema: Results of the EDEMA1 Study

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DX-88, a novel recombinant protein that is a highly specific, potent kallikrein inhibitor, is currently in advanced clinical development for the treatment of hereditary angioedema (HAE). This report summarizes the final results of EDEMA1, one of 3 phase II trials in HAE.

EDEMA1 was a randomized (10:2 ratio of DX-88 to placebo), double-blind, placebo-controlled, dose-escalation trial designed to evaluate the safety and efficacy of DX-88 in patients with acute HAE attacks. A total of 48 patients were randomized to 4 dose groups (5, 10, 20, and 40 mg/m²) and placebo. Primary efficacy endpoint was the proportion of patients with significant symptom improvement by 4 hours post-dosing.

Within 4 hours of dosing, 72.5% (29/40) of patients responded to DX-88, vs 25% (2/8) to placebo ($P=0.017$). No apparent dose-response relationship was observed. Responses were seen at all anatomic sites of attack, including abdominal, laryngeal, and peripheral locations. There was no difference in the proportion of patients responding at any of the sites. DX-88 had a rapid onset of action, as early as the end of infusion. The Kaplan-Meier estimate for the median time to significant improvement was 70 min for the DX-88-treated patients, vs 246 min for placebo ($P=0.355$, Log-Rank test). The DX-88-treated groups also showed more improvement in other secondary efficacy endpoints (eg, time to onset of resolution or time to complete resolution of attack) compared to placebo, but the differences were not statistically significant. The safety profile for DX-88 was similar to that for placebo, and DX-88 was generally well tolerated. No dose-related adverse events were recorded; only 1 drug-related serious adverse event (infusion-related rhinitis) was observed. There has been no evidence to date for anti-DX-88 IgG antibody formation in any of the patients at 4 to 6 weeks post-treatment in EDEMA1 and in the other phase I and II clinical trials with DX-88 in which immunogenicity was measured.

The data support the conclusion that DX-88 is rapidly effective in treating acute attacks of HAE, with a good risk-benefit ratio. EDEMA1 is the first US placebo-controlled clinical trial of a recombinant protein that has been successful in treating acute attacks of HAE.

Molecular correction of C1 inhibitor genomic DNA

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Hereditary angioedema (HAE) is the clinical expression of C1 inhibitor (C1INH) gene mutations causing decreased functional levels of C1INH. Many of these mutations have been identified as point mutations that result in missense or nonsense changes in C1INH. Chimeraplasty has been reported to be capable of correcting DNA point mutations at the genomic level. We therefore investigated whether chimeraplasty could generate sequence specific changes in the C1INH gene.

C1INH specific chimeraplasts were designed to include a single mutation based on human and murine C1INH sequences. Human HepG2 cells were transfected with the chimeraplast, and genomic DNA isolated at various times following transfection. Mice were injected with chimeraplasts *in vivo*, and at various times sacrificed and genomic DNA isolated from their livers. Sequence-specific DNA correction was assessed by single nucleotide primer extension (SNuPE).

We observed sequence-specific conversion of genomic DNA following chimeraplasty in both cultured HepG2 cells as well as in the livers of mice injected with the chimeraplasts. The efficiency of DNA conversion was variable; however, the changes were long-lasting. The introduction of novel rare mutations in the normal C1INH gene provides strong evidence that chimeraplasty resulted in genomic conversion.

Patients with HAE are heterozygous for their C1INH mutation. Thus, C1INH levels at birth and in childhood are often near 50% of normal but fall over time as the consumption of C1INH outstrips its ability to be synthesized by the single normal gene. Correction of the genetic abnormality in even a minority of cells holds the potential to increase C1INH levels above the threshold below which patients are at risk of swelling. Although improvements in the reliability and efficiency of the technique are necessary, chimeraplasty may provide a unique approach to treating HAE for patients with C1INH point mutations.

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