SHORT REPORT

Intravenous immunoglobulins containing antibodies against β-amyloid for the treatment of Alzheimer's disease

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Objective: Active or passive immunisation can mitigate plaque pathology in murine models of Alzheimer's disease (AD). Recently, it has been shown that antibodies against β-amyloid (Aβ) are present in human immunoglobulin preparations (IVIgG), which specifically recognise and inhibit the neurotoxic effects of Aβ. This study reports the results from a pilot study using IVIgG in patients with AD.

Methods: Five patients with AD were enrolled and received monthly IVIgG over a 6 month period. Efficacy assessment included total Aβ/$Aβ_{1–42}$ measured in the CSF/serum as well as effects on cognition (ADAS-cog; CERAD) at baseline and at 6 months following IVIgG.

Results: Following IVIgG, total Aβ levels in the CSF decreased by 30.1% (17.3–43.5%) compared to baseline ($p<0.05$). Total Aβ increased in the serum by 233% ($p<0.05$). No significant change was found in $Aβ_{1–42}$ levels in the CSF/serum. Using ADAS-cog, an improvement of 3.7 ± 2.9 points was detected. Scores in the MMSE were essentially unchanged (improved in four patients, stable in one patient) following IVIgG compared to baseline.

Conclusion: Although the sample size of this pilot study is too small to draw a clear conclusion, the results of this pilot study provide evidence for a more detailed investigation of IVIgG for the treatment of AD.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder with a devastating prognosis. Recently, active immunisation against β-amyloid (Aβ) in preclinical studies using transgenic animal models resulted both in a reduction of Aβ in the cerebrospinal fluid (CSF) and plaque burden.[1 2] The change in Aβ plaque burden was also associated with restored cognitive function in some of these transgenic animals.[3 4] The pathophysiological mechanisms of Aβ removal from the brain after vaccination are still unclear, but may involve microglial-mediated phagocytosis or passage/transport of soluble Aβ into the plasma with a subsequent antibody-mediated degradation.[5] Similar results were obtained in animal studies with passive immunisation using monoclonal antibodies against Aβ.[6] Polyclonal antibodies against Aβ have been detected long before these animal studies in humans; however, their function in AD and non-diseased humans as well as their role in Aβ-degradation were unknown.[7] The detection of antibodies against Aβ in human immunoglobulin preparations (IVIgG) has made them readily available for further investigations.[8] Research into their function have shown that there is a narrow and specific epitopal recognition of Aβ peptides and that they are able to reduce Aβ toxicity and inhibit Aβ fibrillation.[9] We investigated whether treatment using IVIgG, based on the findings above, may have an impact on Aβ levels in the CSF and serum of AD patients.

PATIENTS AND METHODS

Using a prospective, clinical trial design, the safety and preliminary efficacy of IVIgG treatment was evaluated. Each patient received IVIG (Octagam®, Octapharma, Langenfeld, Germany) at a total dose of 0.4 g/kg body weight on three consecutive days every 4 weeks over 6 months. Six individuals were recruited from specialised outpatient clinics for cognitive disorders (table 1). One patient was excluded from the analysis as he refused to get a lumbar puncture at the end of the study. Patients were included who met the criteria for either 'clinically probable' or 'clinically possible' AD according to the National Institute of Neurological and Communication Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria.[10] All AD patients had physical and neurological examinations, neuropsychological testing, as well as laboratory studies and brain imaging to exclude reversible causes of dementia.

All subjects were allowed to continue antidementia medications if stable for at least 6 months before study entry (table 1). No change was allowed during the trial.

Concomitant medications, vital signs, and adverse events were recorded at baseline and at every visit; laboratory tests, electrocardiograms, and physical examinations were performed at the screening and at every subsequent visit. Patients and their relatives were interviewed at each visit to evaluate side effects.

Efficacy measure for this study was the change in Aβ levels in the CSF and serum at 6 months compared to baseline levels. In addition, efficacy was measured by neuropsychological testing, which included the ADAS-cog and the CERAD neuropsychological test battery.

CSF samples were obtained at baseline and at 6 months. It was taken in the morning by lumbar puncture at the L3/L4 or L4/L5 interspace after obtaining appropriate informed consent. CSF white blood cell count and protein levels were determined by standard procedures. No contamination by erythrocytes was seen in any of the samples. Aliquots were then stored at −80℃ until biochemical analysis. Albumin and IgG concentrations were determined in the serum and CSF by immunoprecipitation nephelometry. The CSF albumin/serum albumin quotient was used to evaluate the integrity of the CSF blood barrier. All CSF samples had standard laboratory values within normal ranges.

The Aβ antibody ELISA was performed as described previously.[11] The measurement of total Aβ and $Aβ_{1–42}$ was performed using a sandwich ELISA with commercially available antibodies against $Aβ_{1–40}$ and $Aβ_{1–42}$ (21F12).

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; CSF, cerebrospinal fluid; IVIgG, human immunoglobulin preparations; MMSE, Mini-Mental State Examination

Table 1 Clinical characterisation of included patients

	Age	Sex	Disease duration (y)	Antidementia medication	Duration of treatment (months)
Patient 1	64	Male	4	Donepezil 10 mg	41
Patient 2	55	Female	2	Donepezil 10 mg	22
Patient 3	62	Female	4	Donepezil 10 mg	38
Patient 4	56	Male	5	Rivastagmine 12 mg	49
Patient 5	55	Male	3	Donepezil 10 mg	32

Before screening, written informed consent was obtained from all individuals. The clinical trial protocol was approved by the local ethical committee of the Philipps-University, Marburg.

Statistical analysis

Median levels of anti-Aβ titres were compared at baseline and at 6 months in the CSF and serum by using a Wilcoxon non-parametric test with a p-value of 0.05 regarded as significant. All statistical analyses were performed using SPSS computer software for Windows (Version 11.0).

RESULTS

The demographic and baseline values of the patients enrolled in the study are presented in table 1.

The concentration of total Aβ in the CSF decreased in all patients following 6 months of treatment (mean: 327.5 pg/ml) compared to baseline (mean: 467.0 pg/ml). The decrease was between 17.3–43.5% (mean: 30.1%) of baseline values ($p<0.05$; table 2). No difference in the concentration of $A\beta_{1–42}$ was detectable in the CSF after 6 months.

In the serum, concentration of total Aβ increased with a mean value of 558.2 pg/ml compared to 240.4 pg/ml at baseline ($p<0.05$). No difference in the concentration of $A\beta_{1–42}$ was detectable in the serum after 6 months.

On the ADAS-cog a slight improvement was observed on neuropsychological testing at 6 months in all patients except one where the score did not change between baseline and at 6 months (table 3). A mean improvement of 3.7 ± 2.9 points was calculated. Remarkably, no patient deteriorated. Similar findings were observed for the Mini-Mental State Examination (MMSE). Visual construction abilities, which often fail early in the course of AD, were improved in three of these patients and remained unchanged in two.

This study was completed by all patients. No serious adverse events associated with IVIgG were noted during the clinical trial (one patient was admitted to the hospital because of confusion 2 weeks following IVIgG but it was resolved within a few days). In three patients headaches were reported, which fulfilled the criteria for tension headache. The duration of the headaches were less than 1 day and without any further neurological signs. One patient experienced a tooth infection during the trial; however, this was not directly linked to the treatment as a similar tooth infection occurred twice before the initiation of the study. There were no clinically relevant changes in blood pressure, heart rate, and electrocardiogram findings. There were no clinically relevant differences in haematological or biochemical laboratory test values.

DISCUSSION

In this study we evaluated the effects of IVIgG in patients with AD. In earlier studies we have demonstrated the presence of antibodies directed against Aβ in human IVIgG preparations. These antibodies selectively target Aβ and are capable of antagonising the potential neurotoxic effects of Aβ as well as its fibrillisation — the prerequisite for plaque formation.[8 9 11] The results from the current pilot study clearly show a reduction of total Aβ in the CSF following treatment with IVIgG. In the serum, total Aβ levels were increased similar to earlier findings from studies using transgenic mice expressing a V717F mutation in APP associated with AD.[12] No significant change in the CSF or serum of $A\beta_{1–42}$ were observed, although in some patients a change towards an increased efflux of $A\beta_{1–42}$ may have taken place. In an earlier study using IVIgG in non-demented patients, we had detected an increase of peripheral $A\beta_{1–42}$.[8] The question of whether immunisation may have an effect on plaque burden or may result in a change of the dynamics of Aβ as described previously remains unsolved.[13] Currently, no definite statement on the effects of IVIgG on $A\beta_{1–42}$ is feasible.

In our study we found a reduction in the CSF of total Aβ of 17.3–43.5%. No data are available on the quantitative decrease of Aβ concentration necessary to reduce Aβ deposition. Therefore, one can only speculate whether the observed reduction may have an impact on plaque formation. Further studies, including careful dose studies in animals, are necessary. From earlier studies, however, some information can be obtained. First, a relatively modest Aβ clearance already reduced memory impairment in transgenic mice expressing AD mutations.[3 4] Second, an increase in Aβ concentration of approximately 1.5 times in familial AD patients, because of mutations in the APP gene, shifts the

Table 2 Concentrations of total Aβ ($A\beta_{tot}$), $A\beta_{1–42}$ and antibodies against Aβ (Aβ-Ab) in the CSF and serum at baseline and 6 months following IVIgG

	CSF						Serum			
	Baseline			6 months			Baseline		6 months	
	$A\beta_{tot}$	$A\beta_{1–42}$	Aβ-Aβb	$A\beta_{tot}$	$A\beta_{1–42}$	Aβ-Aβb	$A\beta_{tot}$	$A\beta_{1–42}$	$A\beta_{tot}$	$A\beta_{1–42}$
Patient 1	427.2	130.2	0.32	241.4	133.2	0.37	292.1	63.2	624.3	42.1
Patient 2	408.0	146.3	0.12	295.6	151.3	0.56	147.2	63.1	616.2	67.1
Patient 3	418.8	115.2	0.06	319.8	130.2	0	192.7	58.6	592.7	40.3
Patient 4	532.2	122.2	0	326.8	125.2	0.11	183.4	65.3	379.1	78.2
Patient 5	549.0	147.3	0.66	453.8	124.2	0.49	386.5	64.3	580.2	86.1
Mean	467.0*	132.2†		327.5*	132.8†		240.4*	62.9†	558.2*	62.6†

*$p<0.05$; †$p>0.05$. One patient was excluded from the cerebrospinal fluid (CSF) and serum analysis as he refused to permit the final CSF withdrawal; however, he did finish the study.

Table 3 Results of the neuropsychological testing

	ADAS-cog*		MMSE		Visuoconstruction†	
	Baseline	6 months	Baseline	6 months	Baseline	6 months
Patient 1	34	32	16	20	25	50
Patient 2	23.3	16.3	23	25	50	75
Patient 3	47	41	11	12	0	25
Patient 4	23.6	20.3	25	26	100	100
Patient 5	29	29	22	22	75	75

*Lower values indicate better performance; †Values are the percentage of correct drawn copies. MMSE, Mini-Mental State Examination.

disease onset earlier by several decades. It can be assumed that already small changes in Aβ concentrations in the CSF may have an impact on Aβ deposition and plaque development.[14]

In addition to the CSF markers used in this study, we evaluated the effects on cognitive deficits in AD patients using several established neuropsychological test b batteries. ADAS-cog, which is a commonly used cognitive measure test to evaluate AD patients in clinical studies, showed a modest improvement following 6 months of IVIgG. Evidence from longitudinal studies suggests that the mean level of decline in ADAS-cog over 1 year for untreated AD patients will be approximately 7–11 points and approximately 4–6 points in patients treated with cholinesterase inhibitors.[15] In contrast, a mean improvement of 3.7 points above baseline was observed in this study after 6 months. In addition, other cognitive test scores also improved or were stable at 6 months. Whether these changes following IVIgG may represent a significant stabilisation of the disease or even an improvement in cognitive function cannot be derived from this study because of the small number of patients studied and the potential for considerable variance in cognitive test scores. In addition, the study was not blinded and was not tested against placebo and therefore a bias cannot be excluded. Studies including a larger number of patients, a placebo control, as well as a longer observation period, will be necessary.

In conclusion, the application of IVIgG in AD patients in this setting was well tolerated; however, much more exposure is necessary before a definitive statement about safety can be made. Furthermore, IVIgG caused a reduction of Aβ concentration in the CSF and may also have had a beneficial effect on cognitive functioning in these AD patients. Determining whether passive immunisation with IVIgG varies, stabilises, or significantly delays the cognitive decline in AD will depend upon more detailed clinical assessments over a larger period of time. The results of this pilot study provide further evidence suggesting a more detailed investigation of IVIgG and passive immunisation for the treatment of AD.[5]

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