Eur Arch Psychiatry Clin Neurosci (1991) 240:363–366

European Archives of Psychiatry and Clinical Neuroscience

Integrity of the Blood-CSF Barrier in Dementia of Alzheimer Type: CSF/Serum Ratios of Albumin and IgG

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Received August 29, 1990

Summary. Serum and cerebrospinal fluid (CSF) from 25 patients with dementia of Alzheimer type (DAT) and 25 controls were assayed for concentrations of albumin and IgG. The severity of dementia was rated with the Mini Mental State Examination. The CSF/serum ratio for albumin and IgG as well as the IgG index were used to evaluate blood-CSF barrier function in the respective groups. The control group was matched for age, sex and the indirect alcohol parameters, mean corpuscular volume and gamma-glutamyltranspeptidase. There were no signs of dysfunction of the blood-CSF barrier for proteins or signs of local synthesis of IgG in the central nervous system (CNS) of the demented patients. The permeability of the blood-CSF barrier appeared to be unrelated to dementia severity. The data do not support the hypothesis that a pathological leakage through the blood-CSF barrier facilitates the entry of extraneuronal proteins to the CNS, which might contribute to the pathophysiological process in DAT.

Key words: Albumin – IgG – Dementia of Alzheimer type – Blood-CSF barrier – Cerebrospinal fluid

Introduction

Dementia of Alzheimer type (DAT) is the most common cause of mental deterioration in the elderly [37]. Despite extensive research, the aetiology of the disorder is as yet unknown [18]. Among the various theories about the pathogenesis of DAT, some imply a primary defect of the blood-CSF barrier (BCB) as a cause of the clinical and pathological changes [14]. There are indications that disturbances of the BCB commonly occur in DAT [2, 38, 40]. Indirect evidence for BCB abnormalities exists in the finding of a severe degree of cerebral amyloid angiopathy or cerebrovascular amyloidosis (CAA) around brain vessels of patients with DAT [4, 25]. Widespread amyloid deposition may also be detected in the cerebral and cerebellar cortex [26]. Recently, the precursor protein of CAA has been characterized [15] and cloned [21]. This protein is also expressed [35] and deposited in extracerebral tissues [19]. This has renewed interest in BCB function for proteins. It has been hypothesized that the entry of precursor proteins from serum into the brain might lead to brain amyloid deposition in DAT [19]. Results from studies on serum and cerebrospinal fluid (CSF) proteins to evaluate BCB function are inconsistent [1, 8, 22, 24]. However, none of the previous studies have considered additional effects of alcohol consumption on BCB function [23].

In the present investigation, the BCB function was evaluated by means of the CSF/serum ratio for albumin. The CSF/serum ratio for IgG was used to determine IgG synthesis in the central nervous system (CNS). CSF and serum concentrations of albumin and IgG were measured in carefully diagnosed patients with DAT and in neurological controls without organic disease (see below). Because age, sex and low-dose alcohol consumption [estimated by the indirect alcohol parameters mean corpuscular volume (MCV) and gamma-glutamyltranspeptidase (GGT)], have recently been found to influence BCB permeability [23], the control group was closely matched with respect to these parameters.

Patients and Methods

Twenty-five patients (11 males, 14 females) from the Department of Psychiatry, University of Würzburg, were selected. They had a mean age (± SD) of 69.1 ± 8.3 years (range 52–81). These patients fulfilled the NINCDS/ADRDA criteria for probable DAT [29]. They were hospitalized for a period of 2–3 weeks for diagnostic evaluation. Diagnostic assessment included history, physical, neurological and psychiatric examination and routine laboratory tests (including thyroid hormone levels, vita-

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mine B_{12} and folate). The modified Hachinski ischaemic score (score <4) was used to exclude multi-infarct dementia [33]. Further selection was based on CT scans in all patients showing only cerebral atrophy, ventricular dilatation and no more than one lacunar infarction, if abnormal. In no case were there territorial infarctions. Furthermore, brain mapping of the EEG and acoustically evoked potentials was always consistent with DAT [27], showing a slowing and an anteriorization of the peak frequency and an increase in slow wave activity and typically an anteriorization and decreased amplitude of the P300 wave [28]. In most patients, single photon emission computed tomography with technetium 99m-hexamethylpropylene-amine-oxime (HMPAO-SPECT) was performed and did not reveal signs of multifocal flow deficits. Typically, temporoparietal and/or frontal flow deficits were found [12]. CSF analysis (cytology, isoelectric focusing, microbiological investigations, viral titres) did not reveal signs of a chronic inflammatory process. All patients were investigated with a battery of neuropsychological tests for cognitive functions, language, apraxia, agnosia, visuospatial abilities, mood and behavioural changes (short performance test, subtests from the Wechsler Adult Intelligence Scale, Shopping List Task, Picture Object Test, Mini Mental State Examination (MMSE), Alzheimer Disease Assessment Scale, Brief Cognitive Rating Scale). The estimated duration of the disease was obtained from information provided by relatives or caregivers. The patients had a mean duration of disease ± SD) of 3.1 ± 1.3 years (range: 1–6). For the present investigation, the stage of mental impairment was assessed by means of the MMSE [10]. The patients were mildly to severely demented (mean MMSE score ± SD: 16.2 ± 5.5; range: 9–25). When subdivided into three groups of severity, 8 patients had a severe dementia (0–10 points), 10 had a moderate dementia (11–20 points) and 7 a mild dementia (> 20 points).

As the control group, 25 hospitalized patients (10 males, 15 females) were taken from a large series of subjects [23]. They had a mean age (± SD) of 68.8 ± 5.7 years (range: 61–81). These patients originally were admitted to the hospital with non-specific complaints such as dizziness, headache or neurotic complaints. None of the patients had previously had an operation on or near the CNS, e.g. lumbar disc surgery. Standard neurological examination showed no indication of pathology. The results from a CT scan were within normal limits, except for evidence of brain atrophy in some patients. CSF analysis showed a total white cell count below 5 cells/μl; each CSF sample was clear and colourless, and isoelectric focusing on polyacrylamide gels revealed no evidence of oligoclonal bands.

The CSF was taken during routine lumbar puncture performed for diagnostic purposes in the morning after overnight fasting. Blood samples were drawn at the same time and assayed simultaneously.

For all patients, the following parameters were determined: MCV, serum GGT as objective, albeit indirect, alcohol parameters [23], serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), serum concentration if albumin and IgG, CSF concentration of albumin and IgG. Concentrations of albumin and IgG were determined by an immunonephelometric method using a Beckman nephelometer. The other parameters were determined by routine laboratory analysis.

The function of the BCB was evaluated according to Reiber [32]. Since albumin is exclusively synthesized outside the CNS [9, 32] and must pass through the BCB to enter the CSF, the relative BCB permeability for high-molecular-weight proteins was assessed by the CSF/serum albumin ratio. The CSF/serum IgG ratio was used to determine intrathecal synthesis of immunoglobulins. The IgG index was calculated [36]. The two ratios and the IgG index were used as the primary dependent variables of the present study.

All values were expressed as means ± standard deviation. Non-parametric statistics (Mann-Whitney U-test) were used to evaluate significant group differences between the DAT and control groups. Correlation coefficients were calculated according to Spearman. The level of significance was taken at $P<0.05$.

Results

The clinical characteristics of the DAT patients and control subjects are summarized in Table 1. DAT patients and control subjects were closely matched with respect to age, sex distribution and the indirect alcohol parameters MCV and GGT. They did not differ with respect to GPT and GOT activity.

The dependent variables are summarized in Table 2. The primary dependent variables, i.e. albumin ratio,

Table 1. Characteristics of control subjects and patients with dementia of Alzheimer type

Parameter	Controls	DAT patients
n	25	25
Age (years)	68.8 ± 5.7	69.1 ± 8.3
Range (years)	52–81	61–81
Sex (m/f)	10/15	11/14
Severity of dementia		
MMSE score		16.1 ± 5.5
Mild (frequency)		7
Moderate (frequency)		10
Severe (frequency)		8
Duration of disease (years)		3.1 ± 1.3
Range		1–6
MCV (fl)	90.8 ± 5.5	92.2 ± 4.5
GGT (units/l)	19.4 ± 11.5	14.9 ± 10.8
GOT (units/l)	11.2 ± 6.0	9.6 ± 7
GPT (units/l)	11.9 ± 6.1	12.6 ± 17.3

Values given as mean ± standard deviation; No significant differences were found ($P>0.10$). MMSE, Mini Mental State Examination; MCV, mean corpuscular volume; GGT, gamma-glutamyltranspeptidase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase

Table 2. CSF and serum protein concentrations of control subjects and DAT patients

Parameter	Controls	DAT patients
n	25	25
Albumin ratio	5.16 ± 1.31	6.37 ± 3.04
IgG ratio	2.43 ± 0.64	2.98 ± 1.73
IgG index	0.48 ± 0.08	0.45 ± 0.15
Serum albumin (g/dl)	3.61 ± 0.63	3.99 ± 0.49*
Serum IgG (g/dl)	1.00 ± 0.27	1.28 ± 0.54*
CSF albumin (mg/dl)	18.31 ± 0.44	24.70 ± 1.02*
CSF IgG (mg/dl)	2.37 ± 0.72	3.36 ± 1.47*

Values given as mean ± standard deviation

* Significantly different from control subjects ($P < 0.05$ by Mann-Whitney U-test)

IgG ratio and the IgG index, did not differ significantly between the groups ($P > 0.10$ by Mann-Whitney U-test). However, absolute serum concentrations of albumin and IgG, and similarly, absolute CSF concentrations of albumin and IgG were increased in the DAT patients ($P < 0.05$ by Mann-Whitney U-test). Because of the known dependence of the BCB permeability on age, 11 age-matched pairs were selected from the present sample of patients and controls (mean age ± SD: 71.6 ± 5.9; range: 61–81). Neither the albumin nor the IgG ratio was significantly different in this subset of samples ($P > 0.10$ by *t*-test for paired samples).

The CSF albumin concentration was significantly and positively correlated with the CSF IgG concentration in both the control group ($r = 0.47$, $P < 0.05$) and the DAT patients ($r = 0.88$, $P < 0.0001$). As known from basic physiological studies [13, 32], the albumin ratio was found to correlate strongly and positively with the IgG ratio in the control group ($r = 0.83$, $P < 0.0001$) as well as in the DAT patients ($r = 0.94$, $P < 0.0001$). Only 2 DAT patients had albumin and IgG ratios which were more than 2 SD above the respective means.

The duration of disease did not correlate with the albumin ratio ($P > 0.10$) or the IgG ratio ($P > 0.10$), nor with the CSF concentration of albumin ($P > 0.10$). The MMSE score as a measure of the severity of dementia did not correlate either with the albumin ratio ($P > 0.10$) or the IgG ratio ($P > 0.10$), or with the CSF concentration of albumin or IgG ($P > 0.10$).

Discussion

The increased absolute concentrations of CSF and/or serum albumin and IgG in DAT in the present study agree with the results of some earlier studies [1, 6, 8]. The focus of the present investigation, however, was not on a comparison of the absolute concentrations of these proteins, but on the function of the BCB with respect to high-molecular-weight proteins, which can be reliably assessed by the CSF/serum ratios of albumin and IgG [9, 32]. In the present study, albumin and IgG ratios of the DAT group were not significantly different from controls, indicating an intact BCB function for high-molecular-weight proteins in DAT. The present control group was comparable to the DAT group with respect to all of the factors influencing BCB function; all dependent variables for the control group were in the range of results from previous studies [5, 20] and also comparable to healthy volunteers [3].

The conflicting results of earlier studies may have several reasons:

1. The classification of demented patients as suffering from DAT may not have been accurate enough in earlier studies. Since the introduction of standardized research criteria [29] plus the additional use of CT and neuropsychological tests, diagnostic accuracy can be increased to 87–89% [30, 39].
2. The selection of an adequate control group is difficult in hospitalized patients. In only one study [22] did healthy non-hospitalized subjects volunteer for a lumbar puncture. However, even in these subjects a possible influence of long-standing, low-dose alcohol consumption had not been considered, which has been recognized as contributing to BCB damage [23].
3. Because of the strong influence of the so-far identified covariates of BCB function, a close matching of DAT patients and controls appears mandatory. This had not been achieved in two previous studies [1, 24], precluding a definite interpretation of results.

All these prerequisites have been fulfilled in the present study, therefore validating considerably previous reports of an integrity of the BCB in DAT. Furthermore, no correlation of the BCB permeability with the duration of disease or the severity of dementia could be demonstrated, again indicating the lack of an association with the pathogenetic process in DAT. In addition, positron emission tomography with rubidium 82 or gallium 68-EDTA did not reveal an abnormal BCB permeability in DAT patients [11, 34].

Markers of immunological mechanisms in the CNS, the IgG ratio and the IgG index, were not elevated in the DAT group. Similar results were obtained earlier [1, 8, 20, 22, 24]. However, an immunological mediator, interleukin-1, is elevated in DAT brain [16] and involved in amyloid precursor protein gene expression [17]. Recently, a substance has been identified in human serum and CSF that is immunoreactive with the amyloid precursor peptide and appears to be an IgG. Although the concentration of the immunoreactive material in serum was higher than in CSF, no differences between controls and DAT could be found, thus arguing against an impaired BCB function [31]. However, an intact BCB does not rule out the possibility that substances from the serum penetrate the BCB and interact with neuronal function in the brain.

In summary, the present findings do not support the hypothesis of a compromised BCB function for high-molecular-weight proteins with subsequently increased leakage of serum proteins or of immunologically mediated injury of the CNS as pathogenetic mechanisms in DAT. Further, BCB function is not correlated to the severity of dementia.

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