SHORT REPORT

Levels of CSF prostaglandin E₂, cognitive decline, and survival in Alzheimer's disease

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Background: Although epidemiological, clinical, and experimental evidence indicates that the inducible isoform of cyclooxygenase (COX-2) may be involved in the pathogenesis of several neurodegenerative disorders, the mechanisms whereby COX-2 contributes to Alzheimer's disease are largely unknown.

Objective: To undertake a longitudinal study of CSF levels of a major product of COX activity, prostaglandin E₂ (PGE₂), in relation to cognitive decline and survival in patients with Alzheimer's disease.

Methods: CSF PGE₂ was measured on at least three annual visits in 35 controls and 33 Alzheimer patients (26 necropsy confirmed) who completed the Cambridge cognitive assessment (CAMCOG).

Results: Compared with controls, CSF PGE₂ was higher in patients with mild memory impairment, but lower in those with more advanced Alzheimer's disease. The median survival time of patients with higher initial PGE₂ levels was five years longer than those with lower levels.

Conclusions: COX activity in Alzheimer's disease varies with stage of the disease. PGE₂ levels correlate positively with patient survival. These findings suggest that inhibition of COX activity does not represent a major target for the pharmacological treatment of Alzheimer's disease.

Cyclo-oxygenase (COX) catalyses the first committed step in the synthesis of prostaglandins and is a major target of non-steroidal anti-inflammatory drugs (NSAIDs). COX-2 is the main isoform expressed in inflammatory processes, but also in normal brain, where it is present in discrete neuronal populations mainly distributed in the cortex and hippocampus.¹ Under physiological conditions, COX-2 contributes to synaptogenesis and memory consolidation,² whereas when overexpressed it has been associated with neurotoxicity in acute hypoxia/ischaemia and seizures,³ as well as in neurodegenerative diseases.⁴ Studies of COX-2 expression in brains from people with Alzheimer's disease have produced apparently conflicting results, and both increased and unchanged levels have been reported.^{5–6} In addition, the decreased number of neurones expressing COX-2 in end stage disease suggests that COX-2 expression may vary during the course of the disease.^{7–9} Ex vivo cerebrospinal fluid (CSF) studies may avoid the confounding factors that beset the analyses of postmortem tissue, including the occurrence of terminal systemic infections and variable necropsy delay times. Increased CSF levels of prostaglandin E₂ (PGE₂), a major product of COX-2 activity, have been reported in a small group of patients with probable Alzheimer's disease,¹⁰ consistent with the hypothesis that inflammatory mechanisms might be involved in the disease.

To gain a better insight into the pathological role of COX-2 in Alzheimer's disease, we devised a longitudinal study involving 33 Alzheimer patients and 35 controls, in which we related the CSF levels of PGE₂ to cognitive impairment over at least three years. We wished to test the hypothesis that inflammatory events might primarily be associated with early stages of the disease process. As we followed all patients to death (and most underwent necropsy examinations to confirm the diagnosis), we also tested whether initial levels of CSF PGE₂ predicted subsequent survival in Alzheimer patients.

METHODS

Participants were volunteers in the Oxford project to investigate memory and aging (OPTIMA), a longitudinal observational study established in 1988¹¹ and approved by the Central Oxford research ethics committee. The present study concerned 35 cognitively normal controls and 33 patients who satisfied NINCDS-ADRDA criteria for the diagnosis of probable Alzheimer's disease (table 1).¹² All participants underwent annual medical and neuroimaging assessments for at least three years, and six monthly cognitive assessments using the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).¹³ This included the Cambridge cognitive examination (CAMCOG) and the minimal state examination (MMSE). The CAMCOG learning subscale (out of 17) reflects the severity of episodic memory impairment, the cognitive domain that declines first in most patients.¹⁴ We used the learning subscale scores as proxy of status (control v Alzheimer's disease) and disease progression. We excluded subjects with overt infections, systemic inflammatory conditions, and erythrocyte sedimentation rates (ESRs) over 40 mm/hour. Of the 26 patients who came to necropsy, 25 had CERAD (Consortium to Establish a Registry for Alzheimer's Disease) "definite" and one had CERAD "probable" Alzheimer's disease; six had Braak limbic stage and 20 had neocortical disease. Of the six controls who came to necropsy, four were confirmed as CERAD negative, while two had sparse cortical neuritic plaques. Surviving controls showed no cognitive declines over at least six subsequent years.

CSF samples were centrifuged soon after collection and the supernatants stored at –70°C. We assayed PGE₂ only in CSF samples with no significant blood contamination (<25 red blood cells/μl), using a chemiluminescence enzyme immunoassay (detection limit 1.6 pg/ml; Assay design Inc, Ann

Abbreviations: ADRDA, Alzheimer's Disease and Related Disorders Association; CAMCOG, Cambridge cognitive assessment; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; COX, cyclooxygenase; LMEM, linear mixed effects modelling; MMSE, mini-mental state examination; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke; NSAID, non-steroidal anti-inflammatory drug; OPTIMA, Oxford project to investigate memory and aging; PGE₂, prostaglandin E₂

Table 1 Characteristics of patients with Alzheimer’s disease and controls

	Controls	Patients
Total number of subjects	35	33
Women	17	22
Clinical diagnosis (NINCDS/ADRDA)	35 negative	33 probable
Age at first visit: median (interquartile range, IQR)	70.4 (65.7 to 76.8)	71.0 (64.9 to 77.3)
Pathological diagnosis (CERAD)	4 negative 2 possible	25 definite 1 probable
Learning memory subscale of CAMCOG (median, IQR) first visit	14.0 (13 to 16)*	4.0 (2.8 to 7.3)*
MMSE (median, IQR) first visit	28 (27 to 30)*	19.0 (15.5 to 22.0)*
Sporadic users of low dose aspirin		
Initial visit	6	3
At one or more visits during study period	17	11
Sporadic users of NSAIDs (ibuprofen, diclofenac)	3	1
APOE ₄ allelic frequency	0.10	0.46
CSF PGE ₂ (pg/ml), first visit: median (IQR)	11.0 (6.4 to 14.6)	7.5 (3.3 to 13.0)

* $p < 0.001$, Wilcoxon-Mann-Whitney U test.
ADRDA, Alzheimer’s Disease and Related Disorders Association; CAMCOG, Cambridge cognitive assessment; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; CSF, cerebrospinal fluid; IQR, interquartile range; MMSE, mini-mental state examination; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke; NSAID, non-steroidal anti-inflammatory drug.

Arbor, Michigan, USA). Interference of the CSF matrix with the enzymatic assay was excluded as previously described.¹⁵ Storage time did not relate to CSF PGE₂ levels ($r = -0.01$, $p > 0.5$).

For cross sectional analyses we used a non-parametric test (Wilcoxon-Mann-Whitney U). For longitudinal analyses we used linear mixed effects modelling (LMEM),¹⁶ which included age, sex, and learning subscale scores as fixed factors; the random factors were each individual participant. The LMEMs took into account the longitudinal nature of the data by including an autoregressive correlation function to adjust for serial correlation of errors within participants. Survival analyses used a Cox proportional hazards regression model to test whether age at death related to PGE₂ levels. The Cox proportional hazards regression model co-varied learning subscale or MMSE scores, age at first PGE₂ evaluation, and sex.

RESULTS

PGE₂ levels and cognitive scores at the initial visit are given in table 1. At the second and third study episodes, patients’ MMSE scores declined (median (interquartile range), 13.0 (8.0 to 18.5) and 9 (4 to 16) for the second and third episodes, respectively; paired Wilcoxon-Mann-Whitney test

for first and third study episodes: $V = 421.5$, $p < 0.0001$) but control MMSE scores remained stable (Wilcoxon-Mann-Whitney $V = 100.5$, $p = 0.09$).

The longitudinal analysis by LMEM of the relation between CSF PGE₂ levels and cognitive scores revealed a curvilinear pattern (fig 1A): PGE₂ levels were highest when learning scores were just below the normal range (very early Alzheimer’s disease), but declined with progressive learning impairment (polynomial trends of levels over learning subscale scores: $F = 2.97$, 3/155 df, $p = 0.034$). Patients with learning scores within the top tertile ($>11/17$, $n = 11$), had higher CSF PGE₂ than controls ($t = 2.07$, 38 df, $p = 0.045$). Conversely, patients with learning scores in the lowest tertile ($<6/17$) had significantly lower CSF PGE₂ than controls ($t = -2.19$, 59 df, $p = 0.032$). When patients’ learning scores were in the middle third of the range, their CSF PGE₂ levels were similar to controls ($t < 1$, $p > 0.5$). Co-varying CSF storage times did not affect these results. LMEM modelled the longitudinal nature of the PGE₂ data through a first order autocorrelation structure with a continuous time covariate. This significantly improved the model’s fit ($\phi = 0.35$; Likelihood ratio = 13.05, 1 df, $p = 0.0003$), indicating that relations between PGE₂ levels from individual participants depended on the time interval between observations.

Figure 1 (A) Dependence of the CSF PGE₂ on CAMCOG learning subscale score in all participants (controls, empty circles; patients, filled triangles). A Loess line for the relation is shown. The Loess line fits the data locally using robust non-parametric models. Cerebrospinal fluid PGE₂ levels are given as the natural logarithm of pg/ml values. Non-transformed PGE₂ values varied from 1.6 to 75 pg/ml. (B) Cumulative survival of patients with Alzheimer’s disease with high (dashed line) and low (continuous line) initial CSF PGE₂ levels. Cox proportional hazards regression. Curves are adjusted for sex, age at PGE₂ measurements, and learning subscale scores. CAMCOG, Cambridge cognitive assessment; PGE₂, prostaglandin E₂.

Few subjects used non-aspirin NSAIDs or prophylactic low dose aspirin (150 mg daily) at one or more study episodes (table 1). There was no significant interaction between NSAID use and the polynomial trend of CSF PGE₂ levels over learning subscale scores ($F = 0.88$, 3/151 df, $p = 0.45$) and the curvilinear relation between CSF PGE₂ levels and learning subscale scores was even more significant when these participants were excluded (polynomial trends of PGE₂ levels over learning subscale scores for participants not taking NSAIDs: $F = 3.72$, 3/98 df, $p = 0.014$).

Higher CSF PGE₂ levels at the first visit predicted greater age of death (Cox proportional hazards regression model co-varying learning subscale scores, age at first visit, and sex: $z = 3.29$, $p = 0.001$). To illustrate this, we split the Alzheimer patients into two groups, with CSF PGE₂ levels below or above the median value (9.5 pg/ml). The median learning subscale scores of the two groups were similar (4 and 5 for low and high PGE₂ group, respectively; Wilcoxon-Mann-Whitney $V = 96.5$, $p = 0.30$). The median age of death of the patient group with high PGE₂ levels was approximately five years greater than that of the low PGE₂ group (fig 1B). Patients with higher initial PGE₂ levels had higher MMSE scores (median MMSE for high and low PGE₂: 22 and 16, respectively; Wilcoxon-Mann-Whitney $V = 50.5$, $p = 0.005$), but patients with higher PGE₂ still survived longer even when we co-varied MMSE ($z = 2.33$, $p = 0.023$). When survival analysis was carried out in patients whose initial learning scores were in the lowest tertile, higher PGE₂ levels still predicted longer survival ($z = 2.46$, $p = 0.014$).

DISCUSSION

CSF PGE₂ levels in patients with Alzheimer's disease were high when their short term memory scores were just below those of controls, but were low in later stages of the disease. These findings support the hypothesis that inflammatory processes predominate early in Alzheimer's disease and are consistent with increased intrathecal levels of the pro-inflammatory cytokine TNF α , reported in patients with mild cognitive impairment.¹⁷

Our findings are also consistent with increased CSF PGE₂ levels in probable Alzheimer's disease reported by Montine *et al.*,¹⁰ although the PGE₂ levels reported in that study are higher than those we detected in early disease. This apparent discrepancy might reflect differences in patient characteristics or in assay methods, although the concentration range of our control group was similar to those reported in other studies.^{15 18–20}

A small number of our patients presented with early symptoms and initial learning scores still in the normal range (12–17) before they subsequently progressed to dementia. Their PGE₂ levels were significantly higher than those of the true controls with similar learning scores, thus further supporting the idea of an early inflammatory response in Alzheimer's disease.

CSF PGE₂ levels reflect basal COX activity in hippocampal and cortical neurones^{1 2} as well as inflammation related COX activity occurring in compromised neurones and reactive glia.^{3 4} The curvilinear relation between CSF PGE₂ and dementia severity could reflect an initial increase in inflammatory COX activity, accounting for the rise in PGE₂ observed in early Alzheimer's disease, followed by a progressive neuronal loss resulting in lower basal PGE₂ production. Our study cannot separate the relative contributions of each process at the different stages, but it is consistent with previous necropsy studies reporting a reduction of COX-2 positive neurones in end stage Alzheimer's disease.^{7 8} In a more recent study, the number of neurones expressing COX-2 correlated negatively with the Braak score for A β deposits, while a moderate increase in COX-2

expression was detected in Alzheimer's disease patients with the mildest amyloid stage.⁹

Patients with higher initial CSF PGE₂ levels survived longer. High PGE₂ may reflect a greater survival of COX positive neurones. Alternatively, early inflammatory processes may impede the later progression of Alzheimer's disease. The finding that patients with higher PGE₂ levels survived longer weighs against the idea that PGE₂ or COX activity is neurotoxic. This finding also contrasts with that in sporadic Creutzfeldt-Jakob disease (CJD) where high levels of PGE₂ were associated with a shorter survival time.¹⁸ In sporadic CJD, median PGE₂ levels were about fivefold higher than in Alzheimer's disease,¹⁸ suggesting that inflammatory processes in CJD are more florid and detrimental than they are in Alzheimer's disease.

Retrospective epidemiological studies suggest that prolonged treatment with NSAIDs protects against Alzheimer's disease.²¹ However, if NSAIDs protect against Alzheimer's disease by inhibiting COX activity, it is paradoxical that patients with higher initial PGE₂ levels should survive longer. A likely explanation is that the primary protective effects of NSAIDs are related to mechanisms not involving COX inhibition.²²

In conclusion, our study shows that COX activity in Alzheimer's disease varies during the course of the disease. It does not support the view that increased COX activity is detrimental. Our findings suggest that inhibition of COX activity does not represent a major target for the pharmacological treatment of Alzheimer's disease.

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NEUROLOGICAL PICTURE

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Pudendal nerve compression by pelvic varices: successful treatment with transcatheter ovarian vein embolisation

A 37 year old woman complained of chronic perineal pain and numbness for three years. Physical examination was unremarkable, but perineal neurophysiological testing revealed isolated abnormalities of the left pudendal nerve. The distal motor latency and the left bulbocavernous reflex latency were both lengthened (5.3 ms; normal <3.5 ms and 48 ms; normal <42 ms, respectively). Previous laparoscopy for tubal ligation also described bilateral ovarian varices more prominent on the left side, which were confirmed at pelvic CT (fig 1A).

Diagnosis of Alcock syndrome was rejected because pain was not exacerbated while seated, but rather in the upright position.¹ Although perineal pain has not been reported in pelvic congestion syndrome,² the possibility of venous compression resulting in nerve damage was raised. The patient was then referred to undergo an ovarian phlebography with possible subsequent embolisation.³ The phlebogram disclosed an enlarged left ovarian vein with congestion of the ovarian plexus (fig 1B) and selective left ovarian vein embolisation was performed with coils and glue (fig 1C). Three months later, our patient began to notice marked reduction in perineal pain and numbness. Neurophysiological examination performed eight months after embolisation

Figure 1 37 year old woman with left pudendal nerve compression by pelvic varices successfully treated with transcatheter ovarian vein embolisation. (A) Contrast enhanced CT scan through the ischial spine shows left ovarian varices (arrow). (B) Left ovarian vein phlebography shows dilated ovarian vein with congestion of the ovarian and uterine plexi (arrow) and filling of the pelvic veins across the midline. (C) Following embolisation, control phlebography demonstrates occlusion of left ovarian vein and reduction of pelvic varices (arrow).

demonstrated normalisation of the left pudendal nerve distal motor latency.

This report suggests for the first time the possible compression of the pudendal nerve by pelvic varices, and should be analysed in line with other recently reported nervous compression cases of venous origin.^{4–5} It also demonstrates the dramatic relief obtained after ovarian vein embolisation.

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