Levels of CSF prostaglandin E\(_2\), cognitive decline and survival in Alzheimer’s disease

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ABSTRACT

Objectives: 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 by which COX-2 contributes to Alzheimer’s disease (AD) are still largely unknown. We have performed a longitudinal study of CSF levels of a major product of COX activity, PGE$_2$, in relation to cognitive decline and survival in patients with AD.

Methods: We measured CSF PGE$_2$ on at least three annual visits in 35 controls and 33 AD patients (26 post-mortem confirmed) who completed the Cambridge Cognitive Assessment (CAMCOG).

Results: Compared with controls, CSF PGE$_2$ was higher in patients with mild memory impairment, but lower in those with more advanced AD. The median survival time of patients with higher initial PGE$_2$ levels was five years longer than those with lower levels.

Conclusions: We find that COX activity in AD varies with stage of the disease and that PGE$_2$ 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 AD.
Cyclo-oxygenase (COX), catalyses the first committed step in the synthesis of prostaglandins (PGs) 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.\textsuperscript{1} Under physiological conditions, COX-2 contributes to synaptogenesis and memory consolidation,\textsuperscript{2} whereas when over-expressed it has been associated with neurotoxicity in acute hypoxia/ischemia and seizures,\textsuperscript{3} as well as in neurodegenerative diseases.\textsuperscript{4} Studies of COX-2 expression in AD brains have produced apparently conflicting results and both increased and unchanged levels have been reported.\textsuperscript{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.\textsuperscript{7-9} \textit{Ex vivo} cerebrospinal fluid (CSF) studies may avoid the confounding factors that beset the analyses of post mortem tissue, including the occurrence of terminal systemic infections and variable post mortem delay times. Increased CSF levels of prostaglandin E\textsubscript{2}, a major product of COX-2 activity, have been reported in a small group of patients with probable AD,\textsuperscript{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 AD, we devised a longitudinal study involving 33 AD patients and 35 controls, in which we related the CSF levels of PGE\textsubscript{2} 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. Since we followed all patients to death (and most underwent post mortem examinations to confirm the diagnosis), we also tested whether initial levels of CSF PGE\textsubscript{2} predicted subsequent survival in AD patients.
PATIENTS AND METHODS

Participants were volunteers in the Oxford Project to Investigate Memory and Ageing (OPTIMA), a longitudinal observational study established in 1988 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). 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). This included the Cambridge Cognitive Examination (CAMCOG) and the mini-mental state examination (MMSE). The CAMCOG learning sub-scale (out of 17) reflects the severity of episodic memory impairment, the cognitive domain that declines first in most patients. We used the learning sub-scale scores as proxy of status (control vs AD) 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 autopsy, 25 were Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) “definite” and one was CERAD “probable” AD; six had Braak limbic stage and 20 had neocortical disease. Of the 6 controls who came to autopsy, 4 were confirmed as CERAD negative; 2 had sparse cortical neuritic plaques. All 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 immuno-assay (detection limit: 1.6 pg/ml; Assay design Inc., Ann Arbor, MI). Interference of the CSF matrix with
the enzymatic assay was excluded as previously described.\textsuperscript{15} Storage time did not relate to CSF PGE\textsubscript{2} levels ($\rho = -0.01$, $p > 0.5$).

Cross-sectional analyses used a non-parametric test (Wilcoxon-Mann-Whitney $U$).

Longitudinal analyses used linear mixed effects modelling (LMEM),\textsuperscript{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 auto-regressive correlation function to adjust for serial correlation of errors within participants. Survival analyses used a Cox proportional hazards regression model (CPHR) to test whether age at death related to PGE\textsubscript{2} levels. The CPHR model co-varied learning sub-scale or MMSE scores, age at first PGE\textsubscript{2} evaluation and sex.
RESULTS

PGE$_2$ 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 (8.0–18.5) and 9 (4–16), for second and third episodes, respectively; paired Wilcoxon-Mann-Whitney (WMW) test for first and third study episodes: WMW V=421.5, p<0.0001] but controls’ MMSEs remained stable (paired WMW test for first and third study episodes: WMW V=100.5, p=0.09).

The longitudinal analysis by LMEM of the relationship between CSF PGE$_2$ levels and cognitive scores revealed a curvilinear pattern (Figure 1A): PGE$_2$ levels were highest when learning scores were just below the normal range (very early AD), 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 PGE$_2$ 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 PGE$_2$ than controls (t=-2.19, 59 df, p=0.032). When patients’ learning scores were in the middle third of the range, their CSF PGE$_2$ 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 PGE$_2$ data via 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 relationships between PGE$_2$ levels from individual participants depended on the time interval between observations.

Few subjects used non-aspirin NSAIDs or prophylactic low-dose aspirin (150mg daily) at one or more study episodes (see table 1). There was no significant interaction between
NSAID use and the polynomial trend of CSF PGE₂ levels over learning sub-scale scores (F=0.88, 3/151 df, p=0.45) and the curvilinear relationship between CSF PGE₂ levels and learning sub-scale scores was even more significant when these participants were excluded (polynomial trends of PGE₂ levels over learning sub-scale 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 (CPHR, co-varying learning sub-scale scores, age at first visit and sex: z=3.29, p=0.001). To illustrate this, we split the AD patients into two groups, with CSF PGE₂ levels below or above the median value (9.5 pg/ml). The median learning sub-scale scores of the two groups were similar (4 and 5 for low and high PGE₂ group, respectively: WMW V=96.5, p=0.30). The median age of death of the patient group with high PGE₂ levels was approximately five years greater that that of the low PGE₂ group (Figure 1B). Patients with higher initial PGE₂ levels had higher MMSE scores (median MMSE for high and low PGE₂: 22 and 16, respectively, WMW 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 performed 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 PGE\textsubscript{2} levels in AD patients 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 AD and are consistent with increased intrathecal levels of the pro-inflammatory cytokine TNF-\textalpha, reported in patients with mild cognitive impairment.\textsuperscript{17}

Our findings are also consistent with increased CSF PGE\textsubscript{2} levels in probable AD patients\textsuperscript{10} reported by Montine \textit{et al}., although the PGE\textsubscript{2} 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.\textsuperscript{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 PGE\textsubscript{2} 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 AD.

CSF PGE\textsubscript{2} levels reflect basal COX activity in hippocampal and cortical neurones\textsuperscript{1,2} as well as inflammatory-related COX activity occurring in compromised neurones and reactive glia.\textsuperscript{3,4} The curvilinear relationship between CSF PGE\textsubscript{2} and dementia severity could reflect an initial increase in inflammatory COX activity, accounting for the rise in PGE\textsubscript{2} observed in early AD, followed by a progressive neuronal loss resulting in lower basal PGE\textsubscript{2} production. Our study cannot separate the relative contributions of each process at the different stages, but it is consistent with previous autopsy studies reporting a reduction of COX-2 positive neurones in end-stage AD.\textsuperscript{7,8} In a more recent study, the
number of neurones expressing COX-2 correlated negatively with the Braak score for Aβ-deposits, while a moderate increase in COX-2 expression was detected in AD patients with the mildest amyloid stage.9

Patients with higher initial CSF PGE$_2$ levels survived longer. High PGE$_2$ may reflect a greater survival of COX-positive neurones. Alternatively, early inflammatory processes may impede the later progression of AD. The finding that patients with higher PGE$_2$ levels survived longer weighs against the idea that PGE$_2$ and/or COX activity are neurotoxic. This finding also-contrasts with that in sporadic CJD where high levels of PGE$_2$ were associated with a shorter survival time.18 In sporadic CJD median PGE$_2$ levels were about five fold higher than in AD,18 suggesting that inflammatory processes in CJD are more florid and detrimental than they are in AD.

Retrospective epidemiological studies suggest that prolonged treatments with NSAIDs protect against AD.21 However, if NSAIDs protect against AD by inhibiting COX activity, it is paradoxical that patients with higher initial PGE$_2$ 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 AD 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 AD.
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REFERENCES


Table 1. Characteristics of Alzheimer's disease patients and controls

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<th>Controls</th>
<th>Patients</th>
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<tr>
<td>Total number of subjects</td>
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<td>33</td>
</tr>
<tr>
<td>Females</td>
<td>17</td>
<td>22</td>
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<tr>
<td>Clinical diagnosis (NINCDS/ADRDA)</td>
<td>35 Negative</td>
<td>33 Probable</td>
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<tr>
<td>Age at first visit: median (IQR)</td>
<td>70.4 (65.7 – 76.8)</td>
<td>71.0 (64.9 – 77.3)</td>
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<tr>
<td>Pathological diagnosis (CERAD)</td>
<td>4 Negative</td>
<td>25 Definite</td>
</tr>
<tr>
<td></td>
<td>2 Possible</td>
<td>1 Probable</td>
</tr>
<tr>
<td>Learning memory sub-scale of CAMCOG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR) 1st visit</td>
<td>14.0 (13 – 16)*</td>
<td>4.0 (2.8 – 7.3)*</td>
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<tr>
<td>MMSE (median, IQR) 1st visit</td>
<td>28 (27 – 30)*</td>
<td>19.0 (15.5 – 22.0)*</td>
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<td>Sporadic users of low dose aspirin: initial visit</td>
<td>6</td>
<td>3</td>
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<tr>
<td>at 1 or more visits during study period</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Sporadic users of NSAIDs (ibuprofen, diclofenac)</td>
<td>3</td>
<td>1</td>
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<td>APOE4 allelic frequency</td>
<td>0.10</td>
<td>0.46</td>
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<tr>
<td>CSF PGE2 (pg/ml), first visitmedian (IQR)</td>
<td>11.0 (6.4 – 14.6)</td>
<td>7.5 (3.3 – 13.0)</td>
</tr>
</tbody>
</table>

*p<0.001, Wilcoxon-Mann-Whitney U test
Figure legend

Figures 1

A. Dependence of the CSF PGE_2 on CAMCOG learning sub-scale score in all participants (controls = ○, patients = ▲). A Loess line for the relationship is shown. Loess line fits the data locally using robust non parametric models. CSF PGE_2 levels are given as natural logarithm of pg/ml values. Non transformed PGE_2 values varied from 1.6 to 75 pg/ml.

B. Cumulative survival of AD patients with high (---) and low (—) initial CSF PGE_2 levels. Cox proportional hazards regression. Curves are adjusted for sex, age at PGE_2 measurements and learning sub-scale scores.
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