Short Communication

Different Adenosine A2A Receptor Expression in Peripheral Cells from Elderly Patients with Vascular Dementia and Alzheimer’s Disease

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Abstract. The line between vascular dementia (VaD) and Alzheimer’s disease (AD) is often blurred. In this study we investigated whether adenosine A2A receptor (A2AR) expression can be used to differentiate between VaD and AD. We evaluated the expression of this receptor in the peripheral blood mononuclear cells of patients with VaD, mild cognitive impairment, AD, and controls. We found statistically significant lower levels of A2AR mRNA in VaD compared to AD subjects. These data suggest that A2AR expression may help in the differential diagnosis between VaD and AD.

Keywords: Adenosine receptors, Alzheimer’s disease, biomarker, vascular dementia

Although Alzheimer’s disease (AD) might be best designated as a purely degenerative disease in whose pathogenesis amyloid-β plays a key role, it is acknowledged that in elderly patients (>65 years) there is an increased likelihood of other neuropathological abnormalities including cerebrovascular lesions [1–3]. Over the last years, there has been accumulating evidence that the previously held sharp distinction between AD and vascular dementia (VaD) may not be so clear-cut, especially in old age [4].

VaD is the second most common cause of dementia after AD. The diagnosis of VaD is based on a number of criteria: cognitive deficits, history of stroke and/or focal vascular neurological deficits, and temporal association between stroke and onset of dementia [5]. VaD arises as a consequence of ischemic insults such as hemorrhage and hypoperfusion that trigger neurodegeneration by depriving nerve cells of oxygen and glucose [6, 7]. Such deprivation results in the depletion of nerve cell energy supplies, leading to membrane depolarization, followed by an excessive release of glutamate which activates the N-methyl-D-aspartate receptor (NMDAR). This allows the influx of toxic levels of Ca2+ into nerve cells, which, in turn, activates intracellular calcium-dependent enzymes [8, 9].
The purineribonucleoside adenine (Ado) is a naturally occurringmetabolite that is ubiquitously distributed throughout the body as a metabolic intermediary. Intra- and extracellular Ado levels rise in response to physiological stimuli and with metabolic/energetic perturbations, inflammatory challenges, and tissue injury [10, 11]. The physiological responses to Ado take place as a result of the binding and activation of different transmembrane receptors: the high-affinity A1 and A2A (A2AR) receptors, the low-affinity A2B receptor, or the low-abundance A3 receptor [12].

It has been demonstrated that A2AR is able to prevent amyloid-β-induced synaptotoxicity in animal models and cell cultures [13]. Moreover, A2AR has been shown to control NMDA currents and glutamate outflow in the hippocampus [14, 15].

Contrasting data have been reported so far on the beneficial/detrimental effects of A2AR on brain cells [16]. The blockade of A2AR alleviates the long-term burden of brain disorders such as ischemia, epilepsy, Parkinson’s disease, or AD [14, 17–19]. On the other hand, agonists of A2AR can protect the central nervous system against several insults, including ischemia and excitotoxins [20, 21].

In the periphery, A2AR contributes to coronary endothelial dilatation in mice [22], can inhibit endothelial apoptosis [23], and preserves vascular reactivity following hemorrhagic shock in rats [24].

Finally, increasing evidence supports the notion that A2AR is implicated in the downregulation of inflammation [12, 25].

We recently investigated A2AR gene expression and protein levels in the peripheral blood mononuclear cells (PBMCs) of patients with amnestic mild cognitive impairment (mMCI), multiple cognitive domain MCI (mcdMCI), outright AD, and age-matched healthy controls. We found the highest levels of A2AR in mMCI, suggesting an involvement of the A2AR system in the early stages of AD [26].

The aim of the present study was two-fold: a) to confirm our previous findings in a larger sample of new recruited patients, and b) to determine the expression of the A2AR in the PBMCs from VaD subjects in order to investigate its potential role as an easily accessible biomarker in the differential diagnosis between AD and VaD.

The study involved 40 VaD, 85 AD, 13 mMCI, 58 mcdMCI, and 76 control subjects. The proteins were isolated, RNA was extracted, and real-time PCR was carried out as previously described [26] in 40 VaD, 85 AD, 13 mMCI, 58 mcdMCI, and 76 control subjects. The proteins of 24 VaD, 48 AD, 13 mMCI, 24 mcdMCI, and 21 control subjects were extracted and A2AR levels were measured by western blot as previously described [26].

The statistical analyses were performed by means of the SPSS statistical package (SPSS version 20, Chicago, IL). mRNA and protein levels, expressed as mean ± standard error, were compared across groups by using the one-way ANOVA, with Student’s t-test applied to paired comparisons. A p value <0.05 was considered statistically significant. The predictive efficacy of A2AR was assessed using the area under the curve (AUC) generated by a receiver operating characteristic (ROC) analysis.

We found different A2AR mRNA levels in VaD (1.04 ± 0.34), mMCI (1.42 ± 0.12), control (1.66 ± 0.16), AD (1.92 ± 0.17), and mMCI (3.05 ± 0.92) subjects, with a significant linear increase from VaD to mMCI patients regardless of age and gender (p < 0.001). Comparing the gene expression of each group to controls, we did not find significant differences; on the contrary, the gene expression in the PBMCs of AD and VaD subjects was significantly different (p < 0.001) (Fig. 1). Interestingly, ROC analysis showed that A2AR identifies VaD from a heterogeneous group composed of VaD and AD patients with AUC 0.73 (95% CI 0.63–0.83). Along this line, A2AR density displayed an increased trend from VaD (0.44 ± 0.03) to mMCI (0.59 ± 0.09) subjects, with intermediate levels found in mcdMCI (0.47 ± 0.04), AD (0.49 ± 0.04), and control (0.59 ± 0.04) subjects.

The frequency of ApoE ε4 was in line with previously published data [30, 31]. A2AR gene expression according to NINCDS-ADRE criteria [27]. All AD patients fulfilled the NINCDS-ARDA criteria [28]. A computed tomography or magnetic resonance imaging scan corroborated the diagnosis of dementia. On the basis of their cognitive characteristics, MCI patients were classified as aMCI or mcdMCI. Disease severity was evaluated using Mini-Mental State Examination score. Controls were assessed to exclude the presence of neurological and cognitive disorders of any kind. All participants gave their informed consent to the study, which had been previously approved by the local ethics committee. Blood from all patients and controls was collected between 8 and 9 a.m., after a 6-hour fast. Caffeine consumption was about 80 mg/die (one cup of coffee) or less. Apolipoprotein E (ApoE) genotypes were determined in all samples for which DNA was available [29]. PBMCs were isolated, RNA was extracted, and real-time PCR was carried out as previously described [26] in 40 VaD, 85 AD, 13 mMCI, 58 mcdMCI, and 76 control subjects. The proteins of 24 VaD, 48 AD, 13 mMCI, 24 mcdMCI, and 21 control subjects were extracted and A2AR levels were measured by western blot as previously described [26]. The statistical analyses were performed by means of the SPSS statistical package (SPSS version 20, Chicago, IL). mRNA and protein levels, expressed as mean ± standard error, were compared across groups by using the one-way ANOVA, with Student’s t-test applied to paired comparisons. A p value <0.05 was considered statistically significant. The predictive efficacy of A2AR was assessed using the area under the curve (AUC) generated by a receiver operating characteristic (ROC) analysis.

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Table 1 Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>VaD</th>
<th>AD</th>
<th>aMCI</th>
<th>mcdMCI</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>40</td>
<td>85</td>
<td>13</td>
<td>58</td>
<td>76</td>
</tr>
<tr>
<td>Age (mean ± standard error)</td>
<td>82.6 ± 0.9</td>
<td>78.3 ± 0.6</td>
<td>79.9 ± 2.2</td>
<td>78.6 ± 0.8</td>
<td>80.8 ± 0.8</td>
</tr>
<tr>
<td>MMSE (mean ± standard error)</td>
<td>28.3 ± 1.2</td>
<td>26.0 ± 1.4</td>
<td>28.6 ± 2.4</td>
<td>27.9 ± 0.2</td>
<td>27.9 ± 0.2</td>
</tr>
<tr>
<td>ApoE ε4/ε4</td>
<td>29/10</td>
<td>41/44</td>
<td>9/4</td>
<td>39/18</td>
<td>68/8</td>
</tr>
</tbody>
</table>

This study confirms, in a larger sample of subjects, our previous finding that A2AR expression is upregulated in the peripheral cells of aMCI but not AD subjects, supporting an involvement of the Ado system in the early stages of AD. It also shows that A2AR expression is lower in the PBMCs of subjects with VaD than AD, highlighting its possible relevance as a biomarker that may help differentiate two forms of dementia that are often closely associated. ROC analysis data show that A2AR possesses a moderate degree of sensitivity and specificity for identifying VaD patients from a heterogeneous group composed of VaD and AD patients.

The altered A2AR levels in these two types of dementia could be due to the action of A2AR on the conductance of the NMDAR [14, 15] and on glutamate outflow [32], both of which are important mechanisms in the pathophysiology of VaD [9] but are also recognized as key features of early AD [33].

A2AR represents the main Ado receptor involved in inflammation and it is interesting to note that other inflammatory biomarkers show differences in VaD and AD (e.g., α1-globulin and α2-globulin in the serum [34] and C3a and C4a in the cerebrospinal fluid [35]).

Such evidences underline the complexity of A2AR and suggest that the overall effect of adenosine acting at A2AR results from the interplay of several systems activated by A2AR.

From our results it can be concluded that A2AR may play an important but differential role in both types of dementia: its upregulation in the preclinical stages of AD could counterbalance the existing inflammatory
state and its downregulation in VaD could reflect the effects of A2A_R on the brain vasculature. It can therefore be suggested that A2A_R could serve as a biomarker in the differential diagnosis between VaD and AD.

DISCLOSURE STATEMENT


REFERENCES


