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Short Communication

Different Adenosine A_{2A} Receptor Expression in Peripheral Cells from Elderly Patients with Vascular Dementia and Alzheimer's Disease

Cristina Gussago[a,b], Beatrice Arosio[a,b,*], Martina Casati[a], Evelyn Ferri[a], Federica Gualandris[b], Enzo Tedone[a,b], Paola Nicolini[a], Paolo Dionigi Rossi[a], Carlo Abbate[a] and Daniela Mari[a,b]
[a]*Geriatric Unit, University of Milan, Fondazione Ca' Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy*
[b]*Department of Medical Sciences and Community Health, University of Milan, Geriatrics, Milan, Italy*

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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 A_{2A} receptor ($A_{2A}R$) 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 $A_{2A}R$ mRNA in VaD compared to AD subjects. These data suggest that $A_{2A}R$ 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].

*Correspondence to: Beatrice Arosio, Geriatric Unit, Department of Medical Sciences and Community Health, University of Milan, Fondazione Ca' Granda, IRCCS Ospedale Maggiore Policlinico, Via Pace 9, 20122 Milan, Italy. Tel.: +39 02 55035405; Fax: +39 02 50320735; E-mail: beatrice.arosio@unimi.it.

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 Ca^{2+} into nerve cells, which, in turn, activates intracellular calcium-dependent enzymes [8, 9].

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The purine ribonucleoside adenosine (Ado) is a naturally occurring metabolite 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 A_1 and A_{2A} ($A_{2A}R$) receptors, the low-affinity A_{2B} receptor, or the low-abundance A_3 receptor [12].

It has been demonstrated that $A_{2A}R$ is able to prevent amyloid-β-induced synaptotoxicity in animal models and cell cultures [13]. Moreover $A_{2A}R$ 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 $A_{2A}R$ on brain cells [16]. The blockade of $A_{2A}R$ 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 $A_{2A}R$ can protect the central nervous system against several insults, including ischemia and excitotoxins [20, 21].

In the periphery, $A_{2A}R$ 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 $A_{2A}R$ is implicated in the downregulation of inflammation [12, 25].

We recently investigated $A_{2A}R$ gene expression and protein levels in the peripheral blood mononuclear cells (PBMCs) of patients with amnestic mild cognitive impairment (aMCI), multiple cognitive domain MCI (mcdMCI), outright AD, and age-matched healthy controls. We found the highest levels of $A_{2A}R$ in aMCI, suggesting an involvement of the Ado 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 $A_{2A}R$ 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 aMCI, 58 mcdMCI, and 76 non demented gender-matched controls (Table 1). Subjects were recruited from outpatients attending the Geriatric Unit of the Fondazione IRCCS Ca' Granda of Milan, Italy. VaD was diagnosed according to NINCDS-AIREN 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 aMCI, 58 mcdMCI, and 76 control subjects. The proteins of 24 VaD, 48 AD, 13 aMCI, 24 mcdMCI, and 21 control subjects were extracted and $A_{2A}R$ 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 $A_{2A}R$ was assessed using the area under the curve (AUC) generated by a receiver operating characteristic (ROC) analysis.

We found different $A_{2A}R$ mRNA levels in VaD (1.04 ± 0.14), mcdMCI (1.42 ± 0.12), control (1.66 ± 0.16), AD (1.92 ± 0.17), and aMCI (3.05 ± 0.92) subjects, with a significant linear increase from VaD to aMCI 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 $A_{2A}R$ 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, $A_{2A}R$ density displayed an increased trend from VaD (0.44 ± 0.03) to aMCI (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 $\varepsilon 4$ was in line with previously published data [30, 31]. $A_{2A}R$ gene expression

Table 1
Participant characteristics

	VaD	AD	aMCI	mcdMCI	CT
No. of participants	40	85	13	58	76
Age (mean ± standard error)	82.6 ± 0.9	78.3 ± 0.6	78.9 ± 2.2	78.6 ± 0.8	80.8 ± 0.8
MMSE (mean ± standard error)	18.3 ± 1.2	18.6 ± 0.9	26.0 ± 1.4	24.8 ± 0.6	27.9 ± 0.2
ApoE ε4-/ε4+	29/10	41/44	9/4	39/18	68/8

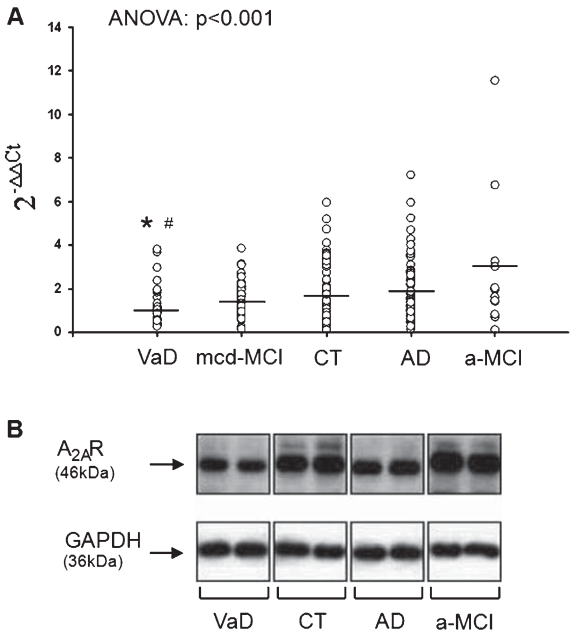

Fig. 1. A) Scatter plot of $A_{2A}R$ mRNA levels in peripheral blood mononuclear cells (PBMCs) from vascular dementia (VaD), multiple cognitive domain MCI (mcdMCI), control (CT), Alzheimer's disease (AD), and amnestic mild cognitive impairment (aMCI) subjects quantified by means of the $2^{-\Delta\Delta Ct}$ method (the lines represent the mean value for each group). $^{*}p < 0.001$ versus AD; $^{\#}p < 0.05$ versus aMCI. B) Representative picture of the western blot analysis of the $A_{2A}R$ densities in PBMCs extracts, run in duplicate, from one subject respectively of VaD, CT, AD, and aMCI groups.

and receptor density were similar in both ε4 carriers and non-carriers in each group of subjects, indicating that the ε4 allele does not participate in the modulation of this gene (data not shown).

This study confirms, in a larger sample of subjects, our previous finding that $A_{2A}R$ 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 $A_{2A}R$ 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 $A_{2A}R$ possesses a moderate degree of sensitivity and specificity for identifying VaD patients from a heterogeneous group composed of VaD and AD patients.

The altered $A_{2A}R$ levels in these two types of dementia could be due to the action of $A_{2A}R$ 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].

The lower $A_{2A}R$ expression in VaD compared to AD subjects seems to suggest a differential role of the Ado system in these dementias.

$A_{2A}R$ 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]).

On the other hand, the $A_{2A}R$ levels reduction in VaD could be a defense mechanism since it has been demonstrated that pharmacologic inactivation or genetic deletion of $A_{2A}R$ reduces neuronal injury after global and focal cerebral ischemia in many animal models [19, 36, 37]. Moreover there are some very robust studies showing that Ado receptors control the dynamics of the brain vasculature [38].

Such evidences underline the complexity of $A_{2A}R$ and suggest that the overall effect of adenosine acting at $A_{2A}R$ results from the interplay of several systems activated by $A_{2A}R$.

We found a significant difference across groups in mRNA levels but only a trend in receptor densities. This could be due to the fact that the number of specimens analyzed by western blot was less than that analyzed by qPCR. Moreover mRNA steady-state levels do not always strictly correlate with protein levels since post-transcriptional changes work together with transcription to achieve the final expression pattern [39, 40].

From our results it can be concluded that $A_{2A}R$ 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 $A_{2A}R$ on the brain vasculature. It can therefore be suggested that $A_{2A}R$ could serve as a biomarker in the differential diagnosis between VaD and AD.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2008).

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