Blood brain barrier permeable β-blockers association with Alzheimer's disease cerebrospinal fluid biomarkers levels in non-demented individuals

Mariateresa Buongiorno,[1,2†] Gonzalo Sánchez-Benavides,[3,4,5†], Clara Marzal-Espí,[6] Darly Milena Giraldo,[1,2] Jerzy Krupinski,[6,7,8] Natalia Cullell,[7] Oriol Grau-Rivera[3,4,5,9], Marc Suárez-Calvet[3,4,5,9], Juan Domingo Gispert,[3,4,10] Alex de la Sierra,[7,11] for the Alzheimer's Disease Neuroimaging Initiative* and the European Prevention of Alzheimer's Disease (EPAD) Consortium.

[†]These authors contributed equally to this work.

Author affiliations:

1 Neurology Department, Vall d'Hebron University Hospital, Barcelona, 08035, Spain

2 Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute, Barcelona, 08035, Spain

3 Barcelonaβeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Barcelona 08005, Spain

4 Hospital del Mar Research Institute, Barcelona 08003, Spain

5 Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid 28029, Spain

6 Department of Neurology, Fundació Assistencial Mútua Terrassa, Terrassa 08221, Spain

7 Fundació per a Docència i Recerca, Mútua Terrassa, Terrassa 08221, Spain

8 Faculty of Science and Engineering, Department of Life Sciences John Dalton Building, Manchester Metropolitan University, Chester Street, Manchester, M1 5GD UK

9 Servei de Neurologia, Hospital del Mar, Barcelona 08003, Spain

10 Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid 28029, Spain

11 Internal Medicine Department, University Hospital Mutua de Terrassa, University of Barcelona, Terrassa 08221, Spain

Correspondence to: Mariateresa Buongiorno

Department of Neurology, Vall d'Hebron University Hospital, Pg. de la Vall d'Hebron, 119, 08035 Barcelona, Spain

E-mail: mtbuongiorno75@gmail.com

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ABSTRACT (100 words)

β-blockers that easily cross the blood-brain barrier (BBB) seem to diminish the risk of Alzheimer's disease (AD), hypothetically facilitating waste clearance. However, their effect on AD pathophysiological markers is unknown. We compared CSF AD core biomarkers levels among non-demented individuals taking low, intermediate or high BBB permeable β-blockers in two samples (ADNI:*n*=216; EPAD:*n*=79). We found that CSF amyloid-β (Aβ) levels were higher in individuals taking highly permeable β-blockers in the ADNI sample. This result was not replicated in EPAD, in which the diminished levels of pTau181 and tTau were observed. These preliminary data suggest that β-blockers may impact AD pathophysiology.

INTRODUCTION

Recent epidemiological research conducted in almost 70.000 individuals reported compelling evidence suggesting that those β-blockers (β-adrenergic antagonists) that easily cross the blood-brain barrier (BBB) diminish the risk of Alzheimer's disease as compared to less permeable β-blockers.[1] Such protective effect seems to be dose-dependent based on BBB permeability, and specific to Alzheimer's disease, since the associations were not identified when all-cause dementia was used as an outcome. These findings support the hypothesis that highly BBB permeable β-blockers protect against Alzheimer's disease by promoting the clearance of brain metabolite waste. The reduction of norepinephrine signaling in the CNS has been related to a reduction of glial cell volume, which would increase the interstitial space, lowering the resistance to parenchymal flow, and facilitating the clearance.[2] However, there is no evidence of the effect of highly permeable β-blockers on Alzheimer's disease pathophysiological biomarkers. We aimed to further explore the suggested protective effect of highly BBB permeable β-blockers in Alzheimer's disease by studying their effect on Alzheimer's disease cerebrospinal fluid (CSF) core biomarker (amyloid-β-$_{42}$ [Aβ_{42}], phosphorylated tau-181 [pTau181], and total tau [tTau]) in non-demented individuals. We hypothesize that participants treated with highly BBB permeable β-blockers would have lower AD pathology as measured by CSF biomarkers.

METHODS

We leveraged on the data collected in the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the European Prevention of Alzheimer's Dementia (EPAD) Longitudinal Cohort Study[3] (ID NCT02804789), from which we selected non-demented individuals (Clinical Dementia Rating [CDR]<1) treated with β-blockers for at least one year and with CSF biomarkers data available at baseline (ADNI:*n*=216; EPAD:*n*=79). Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI)

database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org. See Supplementary material for further EPAD study details. Following the same approach as Beaman *et al*. [1], we classified β-blockers by their BBB permeability as low [atenolol, bisoprolol, sotalol], intermediate [metoprolol] or high [propranolol, carvedilol]. CSF biomarkers were measured using the Roche cobas Elecsys. Aβ positivity was defined using the cut-off $A\beta_{42} < 976.6$ pg/ml in ADNI [4] and $A\beta_{42} <1000$ pg/ml in EPAD.[3] Participants were classified as cognitively normal (Clinical Dementia Rating (CDR)=0) or mildly impaired (CDR=0.5). EPAD-LCS inclusion and exclusion criteria can be found in Supplementary Table 1. We used linear models with log-transformed CSF biomarkers ($A\beta_{42}$, pTau181, tTau) as dependent variables, β-blockers group permeability as the predictor of interest, and age, sex, and global CDR as covariates. Standardized estimates (β coefficients) and 95% confidence intervals were calculated. The interaction terms β-blocker group*Aβ status, β-blocker group*CDR and β-blocker group**APOE*ε4 status were also explored. We also performed binary logistic regressions to predict Aβ status (+/-) using β-blockers group permeability as the predictor of interest, and age, sex, and global CDR as covariates.

RESULTS

Descriptive data by BBB permeability β-blocker groups is shown in Table 1. We found a main effect of β-blocker permeability groups on CSF $A\beta_{42}$ levels in the ADNI sample (p=0.007) but not in EPAD (p=0.495). Pairwise comparisons in the ADNI sample showed significantly higher levels of CSF $A\beta_{42}$ (indicating less amyloid pathology) in the high permeability β-blocker

group as compared to the low (β= 0.144, 95% CI 0.019–0.269, p=0.007) and intermediate permeability groups (β= 0.165, 95% CI 0.042–0.287, p=0.002), see Figure 1 and Table 2. Regarding CSF pTau181 and tTau, there were not main effect of β-blocker permeability groups in ADNI (p=0.483, p=0.542), but significant differences were found in the EPAD sample (p=0.047, p=0.021). In pairwise comparisons, pTau181 (β=-0.311, CI 95% -0.578–-0.043, p=0.023) and tTau levels (β=-0.303, CI 95% -0.422–-0.063, p=0.018) were lower in the high permeability β-blocker group as compared to the low permeability one.

No significant interactions β-blocker group*Aβ status, β-blocker group*CDR, β-blocker group**APOE*ε4 were observed (p>0.1).

Logistic regressions outcomes showed that taking high permeable β-blockers relates to lower probability of Aβ positivity as compared to taking low permeable β-blockers (OR= 0.312, 95% CI 0.116– 0.843, p=0.022) in the ADNI sample. No significant prediction was observed in the EPAD sample (p=0.470).

DISCUSSION

Our results, although preliminary, cast relevant insights on the mechanisms underpinning the reduction of risk of Alzheimer's disease attributed to highly BBB permeable β-blockers. The main finding suggests that the use of β-blockers with high BBB permeability relates to lower levels of amyloid pathology, as reflected by higher levels of CSF $A\beta_{42}$, and maybe to lower levels of CSF pTau and tTau, although the results were inconsistent across the samples analyzed.

We found higher levels of CSF $A\beta_{42}$ associated to the use of β-blockers that easily pass the BBB (propranolol and carvedilol) as compared to the use of less BBB permeable β-blockers in the ADNI sample. Moreover, the odds of being classified as Aβ+ in this sample is 68.8% lower for participants treated with high permeable β-blockers than those treated with low permeable

drugs. However, this result was not replicated in the EPAD cohort, in which we only observed lower levels of CSF pTau181 and tTau associated to highly permeable β-blockers. While the discrepant results on $A\beta_{42}$ may be explained by lack of statistical power associated to the smaller sample size in EPAD, the findings in pTau and tTau are more intriguing. Although we adjusted for demographic variables and clinical staging, samples were different in age (participants were roughly 5 years older in ADNI) and in the distribution of CDR groups (CDR=0.5 was more frequent in ADNI). Such differences in sample characteristics, and other unknown, may underlie the observed discrepancies. Despite the lack of replication of the main results, we acknowledge the findings observed in ADNI over EPAD due to the larger sample size in ADNI, especially in the high permeability group, which is three times larger. Under this rationale, the pTau and tTau findings in EPAD may be considered as potential Type II errors. However, we think that the possible impact of β-blockers that easily cross the BBB on downstream AD pathology deserves further investigation.

The hypothetical protective role of β-blockers has been suggested to rely on its ability to facilitate the removal of waste proteins by the glymphatic system. The glymphatic system is a sophisticated clearance pathway composed of perivascular spaces, astrocytes, and aquaporin-4 channels that manage brain waste and nutrient delivery. Astrocytes, through their endfeet, surround blood vessels and drive the exchange of CSF with interstitial fluid through aquaporin-4 channels (see [5] for a thorough review). This dynamic flow, which is more active during sleep, removes neurotoxic substances such as Aβ and tau proteins.[6,7] The volume fraction and the tortuosity of the extracellular space is critical for determining the concentration of solutes and limits the rate at which they can arrive and leave the compartment.[5] Moreover, the volume of extracellular space dynamically changes as a function of cell volume and neural activity, being adrenergic signaling an important factor in modulating both cortical neuronal activity and the volume of the extracellular space.[6] Specifically, the stimulation of astroglial β-adrenergic

receptors produces cellular expansion (with increments of up to 30-50% in cell perimeter), and antagonists such as propranolol block this expansion.[8] Thus, both the physiological well-known reduction of adrenergic stimulation during sleep,[9] or pharmacological treatments such as the use of β-blockers, would increase the interstitial space volume, lowering the resistance to parenchymal flow and promoting waste clearance. Therefore, it is plausible that a sustained treatment with propranolol or carvedilol facilitates amyloid clearance and led to higher levels of CSF $A\beta_{42}$ as a result. This finding supports the epidemiological data that highly permeable BBB β-blockers may prevent AD [1] by providing, for the first time, pathophysiological proof.

The results presented here should be taken with caution due to relevant limitations of the study. The main one is the relatively small sample size of the individuals taking highly permeable β-blockers. This limitation is driven by the scarcity of data with both AD biomarkers and information on β-blocker medication available. The small sample sizes analyzed may have contributed to both Type I and Type II errors, as indicated by the divergent results observed between the ADNI and EPAD samples. In addition, in the data used, the prescription reason is in many cases unknown or ambiguous, and related comorbidities (vascular risk factors) have not been considered and may have affected the results. Comorbidities and lifestyle factors (such as sleep quality, exercise, etc.) can affect AD biomarker levels and protein clearance, and may have significantly influenced the observed results, potentially explaining the discrepancies. More thorough studies that account for these variables are needed to clarify their effects. Moreover, most of the participants in the available samples were White/Caucasian (95% in ADNI and virtually 100% in EPAD), which limits the generalizability of the findings. Future research using larger, better phenotyped, and more diverse samples is necessary to confirm the observed beneficial effects of β-blocker that easily pass to the CNS.

The data presented provide preliminary pathophysiological support for the hypothesis of enhanced glymphatic function and improved protein clearance potentially influenced by

norepinephrine signaling, and consequent reduction of astrocytic size, produced by β-blockers able to pass the BBB. Future studies to confirm or refute the observed associations and deepen the knowledge of the underlying mechanisms are warranted. Such knowledge might contribute to the development of new Alzheimer's disease prevention strategies.

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CONFLICT OF INTEREST

Gonzalo Sánchez-Benavides worked as a consultant for Roche Farma, S.A. Marc Suárez-Calvet has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U, Roche Farma, S.A and Roche Sistemas de Diagnosticos, Sociedade Unipessoal, Lda.

Gonzalo Sánchez-Benavides is Editorial Board Member of this journal but was not involved in the peer-review process nor had access to any information regarding its peer-review. All other authors have no conflict of interest to report.

DATA AVAILABILITY

The data used in reparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu), which is available upon request, and from the EPAD LCS data set V.IMI (doi:10.34688/epadlcs_v.imi_20.10.30). EPAD LCS data is publicly available upon request to the EPAD data access committee.

SUPPLEMENTARY MATERIAL

EPAD study details and EPAD-LCS inclusion and exclusion criteria can be found in the supplementary material available in the electronic version of this article.

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Table 1. Descriptive data of the samples by β-blocker permeability groups

	β-blocker permeability groups		
	Low	**Intermediate**	**High**
ADNI	n=80	n=109	n=27
Age, years M±SD	73.8±7.0	75.5±7.5	74.0±7.6
Sex, females n(%)	41(51.3%)	36(33.0%)	15(55.5%)
APOEε4 carriers n(%)	38(47.5%)	50(45.9%)	2(7.4%)
CDR 0.5 n(%)	50(62.5%)	74(67.9%)	14(51.9%)
Aβ+ n(%)	43(53.8%)	71(65.1%)	7(25.9%)
CSF $A\beta_{42}$ pg/ml M±SD	1081±603	1001±574	1501±707
CSF pTau181 pg/ml M±SD	25.6±11.3	28.6±14.2	20.6±12.6
CSF tTau pg/ml M±SD	270±104	294±125	261±101
EPAD	n=58	n=13	n=8
Age, years M±SD	70.1±7.2	68.4±6.5	68.2±6.8
Sex, females n(%)	24(41.4%)	6(46.2 %)	3(37.5%)
APOEε4 carriers n(%)	26(44.8%)	6(46.2%)	2(25.0%)
CDR 0.5 n(%)	27(46.6%)	5(38.5%)	3(37.5%)
Aβ+ n(%)	30(51.7%)	8(61.5%)	3(37.5%)
CSF $A\beta_{42}$ pg/ml M±SD	1200±566	1134±675	1077±416
CSF pTau181 pg/ml M±SD	22.4±9.8	19.0±8.9	15.2±4.8
CSF tTau pg/ml M±SD	248±94	208±93	173±49

APOE: apolipoprotein E; CDR: Clinical Dementia Rating; MMSE: Mini-Mental State Examination; Aβ: Amyloid-beta; Aβ+ was defined as $A\beta_{42}$< 976.6 pg/ml in ADNI and $A\beta_{42}$ <1000 pg/ml.

Table 2. Effect of β-blockers permeability groups on CSF biomarkers

ADNI	Standardized β	95%CI	P value
CSF $A\beta_{42}$			
High vs Low permeability	0.144	0.019–0.269	**0.007**
High vs Intermediate permeability	0.165	0.042–0.287	**0.002**
Intermediate vs Low	-0.021	-0.091–0.050	0.564
CSF pTau181			
High vs Low permeability	-0.010	-0.089–0.070	0.812
High vs Intermediate permeability	-0.037	-0.115–0.040	0.348
Intermediate vs Low	0.028	-0.026–0.081	0.312
CSF tTau			
High vs Low permeability	-0.002	-0.091–0.077	0.962
High vs Intermediate permeability	-0.003	-0.093–0.042	0.458
Intermediate vs Low	0.024	-0.023–0.071	0.313
EPAD	Standardized β	95%CI	P value
CSF $A\beta_{42}$			
High vs Low permeability	-0.047	-0.191–0.097	0.515
High vs Intermediate permeability	0.017	-0.154–0.188	0.848
Intermediate vs Low	-0.063	-0.181–0.053	0.281
CSF pTau181			
High vs Low permeability	-0.311	-0.578–-0.043	**0.023**
High vs Intermediate permeability	-0.159	-0.481–0.162	0.327
Intermediate vs Low	-0.152	-0.376–0.073	0.183
CSF tTau			
High vs Low permeability	-0.303	-0.422–-0.063	**0.018**
High vs Intermediate permeability	-0.137	-0.422–0.148	0.343
Intermediate vs Low	-0.167	-0.362–0.028	0.093

Models were adjusted by age, sex and CDR.

Figure 1. Boxplots showing CSF biomarker levels (adjusted by age, CDR, and sex) by β-blockers permeability groups. Dashed line depicts the threshold for Aβ positivity in each cohort (ADNI < 976.6 pg/ml; EPAD <1000 pg/ml).