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Research article

Amyloid binding properties of curcumin analogues in Alzheimer's disease postmortem brain tissue

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HIGHLIGHTS

- Binding properties of curcuminoids to Aβ containing plaques in the AD postmortem tissue.
- Bisdemethoxycurcumin might be a potential radioligand for Aβ plaque imaging.
- [^{3}H]Bisdemethoxycurcumin accumulation in all layers of cortical gray matter.

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ABSTRACT

The presence of β-amyloid (Aβ) containing plaques in the brain is a hallmark of Alzheimer's disease (AD) and serves as a biomarker for confirmation of diagnosis postmortem. Early diagnosis is of great importance for optimal treatment and for monitoring disease progression in the brain. Highly specific and sensitive biomarkers are thus greatly needed to assess therapeutic efficacy, not only clinically, but also in terms of clearance of histopathological lesions and decelerated neurodegeneration. The objective of the present study was to give more insight into the binding of curcumin analogues, curcuminoids, to Aβ containing plaques in postmortem tissue from AD patients. In vitro autoradiography was utilized to explore affinity and displacement of the curcuminoids; curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and dimethoxycurcumin (DIMC). We found that BDMC had the highest affinity for Aβ containing plaques in cortical AD brain tissue in comparison to other curcuminoids. Subsequently, [^{3}H]BDMC showed significantly higher specific binding in cortical AD brain tissue compared to control subjects. These findings suggest that curcumin analogues, especially BDMC, may serve as a potential radioligands for Aβ plaque neuroimaging.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, which is characterized by the presence of neuritic plaques and neurofibrillary tangles [24]. The plaques are mainly composed of β-amyloid (Aβ) peptides in fibrillar from, generated by proteolytic processing of the larger amyloid precursor protein (APP) causing impairment in neurotransmission and neuronal death [11,18]. Aβ is a 40–42 amino acid long hydrophobic and self-aggregating peptide, which is central to AD pathogenesis [33]. Aβ monomers gradually aggregate into soluble oligomeric assemblies and eventually into insoluble fibrils [11]. Aβ fibrils, the main constituents of senile plaques, which are hallmarks of AD, have a cross-β structure of the peptide chains and bind specific dyes like Congo red and thioflavin-T [17]. There is a great need to reliably diagnose AD at an early stage and to enable non-invasive monitoring of disease progression in the living AD brain. Highly specific and sensitive biomarkers are thus greatly needed to assess therapeutic efficacy, not only clinically, but also in terms of clearance of histopathological lesions and examination and monitoring of biochemistry in vivo. The recent years, several positron emission tomography (PET) radioligands such as [^{11}C]AZD2184, [^{18}F]AZD4694 and [^{11}C]PIB have been used in the assessment of Aβ containing plaques in the living human brain [5,9,15]. Recent development of antibody-based PET radioligands such as mAb158 have also successfully been used in neuroimaging in AD [28].

Curcumin (diferuloylmethane), is a natural polyphenolic compound and the main curcuminoid of the yellow spice turmeric. Curcumin possesses medical qualities including anti-amyloidogenic [10], anti-inflammatory [29], anti-oxidative [21],

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and metal chelating properties [6] that may result in neuroprotective effects. Naturally, curcuminoids are a mixture of three chemical constituents, namely curcumin (75–80%), demethoxycurcumin (DMC) (15–20%) and bisdemethoxycurcumin (BDMC) (3–5%) [1]. Dimethoxycurcumin (DIMC) is a synthetic curcumin analogue with higher metabolic stability than curcumin [1]. Curcuminoids may have a potential role in the prevention and treatment of AD by involvement in inflammation and oxidative processes, and most particularly, the formation of Aβ plaques [32,34]. Studies have shown that curcumin has high affinity to small Aβ species, resulting in inhibition of aggregation and fibril formation both in vitro [25] and in vivo [36]. The lipophilic properties of curcuminoids enable these compounds to cross the blood-brain barrier (BBB) and bind to Aβ. It has been shown that intravenous administration of curcumin for 7 days enhanced clearance of Aβ deposits in APPswe/PS1dE9 mouse brain [10].

In the present study, we examined the effects of different curcuminoids including curcumin, DMC, BDMC and DIMC (Fig. 1) on Aβ containing plaques in the temporal cortex of AD postmortem brain tissue. We found that BDMC had the highest affinity (K_i 1.39 ± 0.38 mM) for Aβ containing plaques in comparison to DMC, DIMC and curcumin. [^{3}H]BDMC showed specific binding in the cortex of AD postmortem brain tissue in comparison to control subjects. Taken together, the competition binding assays and in vitro autoradiography results suggest that the curcuminoid BDMC may potentially be used as a radioligand for Aβ plaque imaging.

2. Material and methods

2.1. Radiosynthesis of radioligands

Radiosynthesis of [^{3}H]AZD2184 was performed at Karolinska Institutet, Department of Clinical Neuroscience. The specific radioactivity of [^{3}H]AZD2184 was 0.78 TBq/mmol and the radiochemical purity was >99%. [^{3}H]Bisdemethoxycurcumin (2.44 TBq/mmol, 99% radiochemical purity) was custom synthesized and purchased from Novandi Chemistry AB (Södertälje, Sweden).

2.2. Human postmortem brain tissue

The temporal cortices from four AD patients and three normal healthy individuals were obtained from the Netherlands Brain Bank, Amsterdam, The Netherlands. Autopsies were performed on donors from whom written informed consent has been obtained either from the donor or direct next of kin. The clinical diagnosis of demented patients was performed according to the NINCDS-ADRDA criteria [20] and the severity of the dementia was estimated according to the Global Deterioration Scale [27]. The control subjects had no known history or symptoms of neurological or psychiatric disorders. The case details of the control subjects and AD patients are listed in Table 1.

Frozen sections, 20 μm, were prepared from the tissue blocks in a cryomicrotome (Leica CM 1860). Sections were thaw-mounted on microscope slides (SuperFrost®Plus, Menzel-Gläser, Germany), air-dried and directly re-frozen in the cryostat. Slides were kept at −20 °C until use.

2.3. [^{3}H]AZD2184 in vitro competition autoradiography

The autoradiographic experiments were performed essentially as described previously [13]. Slides were thawed at room temperature and incubated for 30 min in 50 mM Tris HCl, pH 7.4 (binding buffer) containing 1 nM [^{3}H]AZD2184 (0.78 TBq/mmol). In matching sets of containers containing [^{3}H]AZD2184 in binding buffer, unlabelled AZD2184 was added to displace specific binding. For

Table 1
Case details of AD patients and control subjects.

Autopsy no.	Age at death (ys.)	Sex Female/male	Braak stage	postmortem time (h)	ApoE alleles
AD 1	88	F	4	5.35	3/3
AD 2	91	M	4	4.15	3/3
AD 3	87	F	4	4.00	4/3
AD 4	101	F	4	4.25	3/3
Control 1	73	F	1	5.30	3/3
Control 2	81	F	1	22.15	3/2
Control 3	68	F	2	10.30	3/3

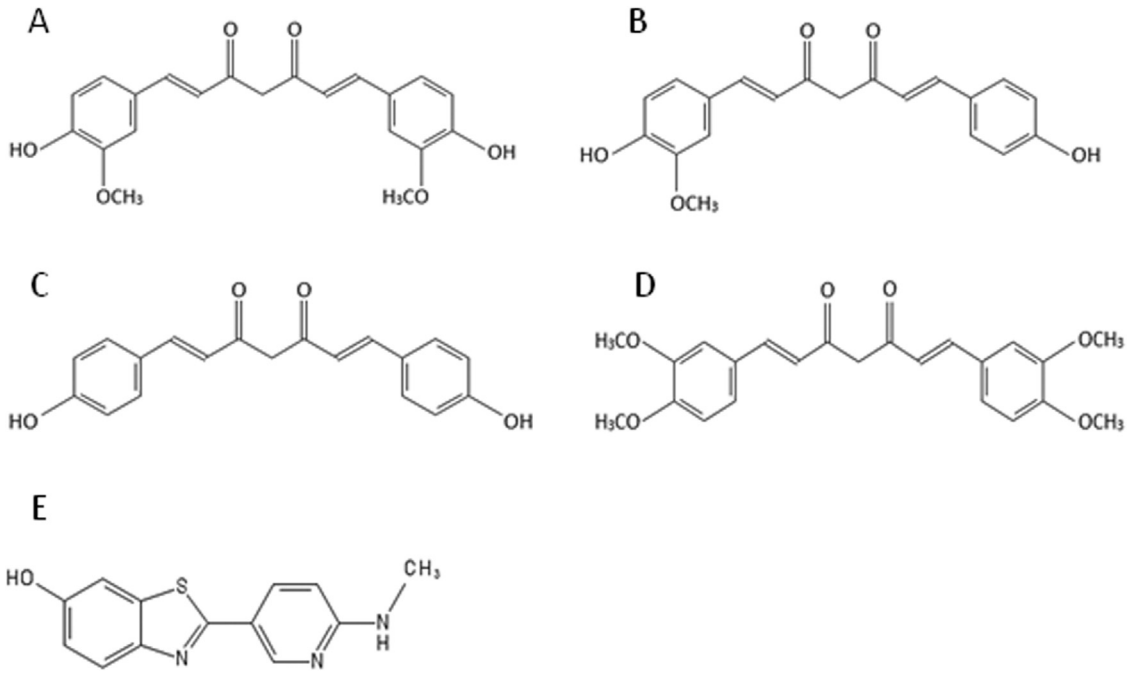

Fig. 1. Chemical structures of curcuminoids. (A) Curcumin, (B) demethoxycurcumin (DMC), (C) bisdemethoxycurcumin (BDMC), D) dimethoxycurcumin (DIMC), E) AZD2184.