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SYNTHESIS OF COPPER COMPLEXES FOR POTENTIAL USE IN THE DIAGNOSIS OF ALZHEIMER'S DISEASE

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A – study design, **B** – data collection, **C** – statistical analysis, **D** – interpretation of data, **E** – manuscript preparation, **F** – literature review, **G** – sourcing of funding

ABSTRACT

Background: Since there is currently no cure for Alzheimer's disease (AD), it is important to develop methods that could contribute to its diagnosis and propose new therapeutic approaches for its treatment. Positron emission tomography (PET) is a medical imaging technique that can be used to diagnose Alzheimer's disease, as some radiotracers have been developed to detect amyloid plaques, one of the hallmarks of the disease. However, the radiotracers already used for the diagnosis of AD have a low half-life, which limits their use over the time and requires the presence of a cyclotron close to the examination site. The use of copper complexes could be a good alternative for the development of new radiotracers, since they can be used in PET imaging and have a longer half-life than the other radiotracers already used for AD diagnosis.

Aim of the study: Design and synthesize copper complexes that could be used as PET radiotracers able to cross the blood brain barrier and detect amyloid plaques.

Material and methods: The first objective was to design a series of new copper complexes with the capacity to target amyloid plaques inside the brain and contribute to the rational synthesis of such complexes. To this end, theoretical models were used to predict the ability of the complex to cross the blood-brain barrier (BBB) which separates brain from the blood and is main gatekeeper for drugs entering the brain. The models employed consider the physicochemical properties of the molecules. Based on these models, one of the molecules was synthesized in ten steps. The key step in the synthetic strategy was the coupling of a monopicolinate-N-alkylated cyclam-based ligand with a moiety capable of recognizing Aβ plaques via a Buchwald-Hartwig coupling reaction. Potentiometric, spectrophotometric and electron paramagnetic resonance spectroscopic studies were performed to determine the structure and thermodynamic stability of the complex. In addition, the ability to target amyloid plaques was evaluated using brain sections from Alzheimer's disease patients and the cytotoxicity was evaluated using human neuronal cells.

Results: A first complex has been designed and synthesized. The physicochemical studies performed indicate that this complex seems to be thermodynamically stable. In addition, cytotoxicity tests on human neuronal cells indicate low toxicity and labeling tests on brain sections from Alzheimer's patients suggest that the complex is capable of recognizing amyloid plaques.

Conclusions: We have developed a novel copper complex that is able to detect amyloid plaques. In the future this molecule could be used in PET and contribute to the diagnosis of Alzheimer's disease. From these results, we also propose to develop a new copper complex for tau PET imaging.

KEYWORDS: Alzheimer, diagnosis, PET, copper complexe

Background

Alzheimer's disease (AD) is the most common form of dementia and one of the leading causes of death in the world [1,2]. It is a neurodegenerative brain disease characterized by memory loss, language problems, loss of sense of time and space, and impaired executive functions. These symptoms result from the progressive alteration or destruction of neurons. The disease is characterized by the presence of two neuropathological lesions: amyloid plaques (extracellular β-amyloid peptide aggregates) and neurofibrillary tangles (intracellular aggregates of hyperphosphorylated tau protein) [3].

AD is a neurodegenerative disorder with no known cure. While treatments such as cholinesterase inhibitors [4] and NMDA receptor antagonists [5] can help manage symptoms and improve quality of life, they do not halt or reverse the underlying progression of the disease. The U.S. Food and Drug Administration recently approved new treatments, including Aducanumab [6] and Lecanemab [7], for mild forms of AD. However, these treatments are not able to cure the disease. Finding a cure for AD is currently the subject of intense research efforts, with a focus on disease-modifying therapies and early detection strategies.

Nowadays, clinical observation and cognitive tests can provide a probable diagnosis to be made, but only in advanced stages of the disease. Only post-mortem tests can provide definitive confirmation. Nevertheless, medical imaging offers the possibility of early diagnosis, not only to establish the correct diagnosis (AD causes symptoms that may be similar to those seen in other diseases such as Lewy body disease, vascular dementia, frontotemporal dementia, etc.), but also to adapt the treatment to the pathology, and thus provide better management of the care [8].

The development of radio-labeled small molecules that can enter the brain and specifically target amyloid plaques for imaging using positron emission tomography (PET) represents a significant breakthrough [9]. PET imaging using radio-labeled small molecules has enabled the detection of amyloid plaques in the brain, which participate to the diagnosis of Alzheimer's disease. Several radiotracers, capable of detecting amyloid peptide and usable in PET have been developed, among them are [^{11}C]-PiB [9], [^{18}F]-Florbetapir [10], [^{18}F]-Flutemetamol [11] and [^{18}F]-Florbetaben [12]. Unfortunately, the main limitation to the use of these radiotracers is that they are labeled with ^{11}C and ^{18}F, with half-lives ($t_{1/2}$) of 20 and 109.7 min, respectively. Thus, these short half-lives make their use limited in the time and require the presence of a cyclotron near to the site of examination.

To overcome this problem, significant research efforts have been devoted to the development of radiopharmaceuticals labeled with copper radionuclides [13] and some copper complexes have been studied for their potential use in the diagnosis of AD. The use of ^{64}Cu could be a promising alternative for the development of PET radiotracers, thanks to its $t_{1/2}$ of 12.7 h. Unfortunately, none of the proposed copper complexes proposed for the diagnosis of Alzheimer's disease have reached the stage of clinical trials. One reason is low brain uptake. This could be probably due to their limited ability to cross the blood-brain barrier. (BBB) [14-16].

The BBB is located between the blood and the brain's extracellular space, in the endothelial cells of brain capillaries. These endothelial cells are distinguishable from systemic endothelial cells due to their increased mitochondrial content, reduced pinocytotic activity, and the presence of tight junctions [17]. These tight junctions effectively seal the intercellular space, restricting paracellular transport. Consequently, most of the molecules should cross the plasma membrane of endothelial cells by passive diffusion or require the presence of transport proteins.

The BBB plays a crucial physiological role in maintaining the homeostasis and integrity of the central nervous system (CNS). Its ability to regulate the passage of substances from the blood into the brain is crucial. However, the BBB's low permeability significantly restricts the passage of diagnostic or therapeutic synthetic molecules to the brain. It is important to emphasize that despite the fact that the BBB is the main obstacle for the entry of synthetic molecules into the brain, most studies related to the development of new molecules leave out the understanding of the molecular mechanisms of synthetic molecules entry into the brain through the BBB.

Aim of the study

Our objective is to propose a rational design for bifunctional copper complexes that can cross the BBB, specifically target amyloid plaques, and be detected by PET. This work could provide new and effective tools for the early diagnosis of AD. To achieve this, we use theoretical calculations for *in silico* prediction to assess BBB permeability, design the new molecules, propose their synthesis, physicochemical characterization, test their ability to specifically recognize amyloid plaques and cytotoxicity.

Material and methods

Chemists can use models to predict the ability of molecules to be absorbed by the organism. These models take into account the physicochemical properties of synthetic molecules (size, hydrogen bonding,

lipophilicity...) and contribute to the rational synthesis of new molecules targeting the brain. Lipinski's rule is a tool used to predict the druglikeness of a synthetic molecule [18,19]. According to this rule, a good absorption and permeability is likely if [20]:

- Molecular weight is lower than 500 Daltons;
- Oil/water distribution coefficient (LogP) is lower than 5;
- Hydrogen bond donors lower than 5 (expressed as the sum of OHs and NHs);
- Hydrogen bond acceptor lower than 10 (expressed as the sum of Ns and Os) .

Log BB is the logarithm of the ratio of a drug's concentration in the brain to its concentration in the blood at equilibrium (Equation 1). This value serves as an index of BBB permeability and is used to assess the potential of drug candidates for CNS indications. A higher log BB value indicates better penetration of the BBB, which means that the compound is more likely to reach therapeutic concentrations in the brain. Log BB values can be derived experimentally either *in vivo* using animals or *in vitro* using cellular BBB models [21].

$$\text{Log BB} = \frac{\text{Concentration in the brain}}{\text{Concentration in the blood}}$$

Equation 1. Equation for log BB

In silico prediction of BBB permeability relies on molecular descriptors such as size, hydrogen bonding and lipophilicity. This method only considers passive diffusion across the BBB. Experimentally determined log BB values are correlated with various descriptors using mathematical models, e.g., multiple linear regression, partial least squares analysis, artificial neural networks, etc.

Several studies have evaluated the ability of molecules to cross the BBB by calculating the Log BB value. For our work, we decided to use the model proposed by Vilar and colleagues [22]. They used 307 drugs targeting the central nervous system to develop their model. In addition, all the log BBs mentioned in their study are for molecules where the value was determined *in vivo*, and the descriptors used in their models can be easily calculated. The model predicted the passage of molecules across the BBB with approximately 80% reliability and proposes a correlation between the theoretical value of log BB and the prediction of whether or not molecules will cross the BBB. According to the authors, if the calculated value of log BB is greater than 0.3, the molecule will easily cross the BBB, while if the value is less than -1, the molecule would be poorly distributed in the brain.

Thus, using the predictive model mentioned before, we propose to design different molecules (ligands) that allow the synthesis of the corresponding copper complexes. These ligands have a part capable of chelating copper and another part capable of recognizing amyloid plaques.

A first complex was synthesized in ten steps. The key strategy of this synthesis is based on the coupling of a cyclic monopicolinate-N-alkylated ligand with a moiety capable of recognizing beta-amyloid plaques by a Buchwald-Hartwig C-N coupling reaction. To define the structure and thermodynamic stability of complex, we have performed potentiometric, spectrophotometric and electron paramagnetic resonance spectroscopy studies in solution. The ability to recognize amyloid plaques was done using sections from brain biopsies from Alzheimer's Disease patients and the cytoxicity test using SH-SY5Y cells [23].

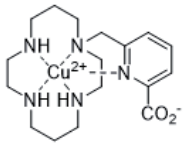
Results

Following our strategy, we designed and synthesized the bifunctional copper complex $[Cu(TE1PA\text{-}ONO)]^+$. This complex was synthesized in ten steps. Solution studies revealed the structure and high thermodynamic stability of $[Cu(TE1PA\text{-}ONO)]^+$, documenting the fact that the attachment of ONO to TE1PA does not weaken the chelating properties of this moiety. To assess the ability of this new complex to specifically detect amyloid plaques, *in vitro* staining of these plaques was performed on brain sections from patients with AD. The staining with the copper complex was compared to the staining with anti-amyloid antibodies. The results show similar distribution and density of amyloid plaques using anti-amyloid antibodies and $[Cu(TE1PA\text{-}ONO)]^+$ on brain sections, indicating that our copper complex is able to detect beta amyloid plaques. Finally, since amyloid plaques accumulate around neurons, the cytotoxicity of the copper complex was tested on human neuronal SH-SY5Y cells. The results obtained indicate a low toxicity towards these neuronal cells [23]. The studies of the capacity of $[Cu(TE1PA\text{-}ONO)]^+$ to cross BBB should be conducted in the future.

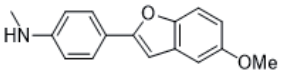
Discussion

The first bifunctional copper complex proposed in our work is the $[Cu(TE1PA\text{-}ONO)]^+$ (23) (Fig. 1). This complex was synthesized by coupling a monopicolinate-N-alkylated cyclam (TE1PA) [24], moiety chelator of copper, with a benzofuran derivative (ONO) [25] capable of recognizing Aβ plaques. Before proposing this bifunctional complex, we studied the ability of $[Cu(TE1PA)]^+$ and ONO, respectively, to cross the plasma membrane. Thus, we studied the intracellular accumulation of both molecules in K562 cells overexpressing or not P-glycoprotein (protein

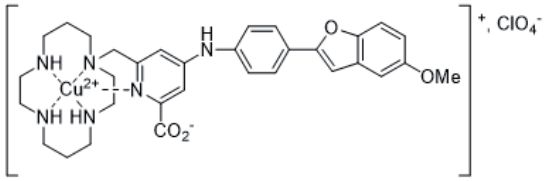
expressed at the BBB level that contributes to barrier functions and is responsible for reducing the penetration of several drugs into the brain). The results obtained suggest that both molecules are able to cross the plasma membrane and are not substrates of P-gp [26,27].



Structure of [Cu(TE1PA)]$^{+}$



Structure of ONO



Structure ofbifunctional copper complex
[Cu(TE1PA-ONO)]$^{+}$$ClO_4^{-}$

Figure 1. Structure of the biofunctional copper complex [Cu(TE1PA-ONO)]$^{+}$(23), synthesized by coupling a monopicolinate N-alkylated cyclam (TE1PA)(24), with a benzofuran derivative (ONO)(25)

[Cu(TE1PA-ONO)]$^{+}$ was designed and synthesized based on theoretical calculations and empirical results. This molecule is capable of recognizing amyloid plaques and exhibits low toxicity to neuronal cells.

Encouraged by previous results, using prediction models, we plan to design and synthesize a new series of copper complexes based on ligands containing a copper chelate moiety coupled with different molecules known to target amyloid plaques (Fig. 2). These new ligands should have a LogBB value greater than 0.3, indicating their potential ability to cross the BBB. In addition, they should satisfy the criteria of the Lipinski rule. The thermodynamic stability of the complexes will be investigated. This point is important to consider when developing metallic complexes for human use, as high stability can prevent

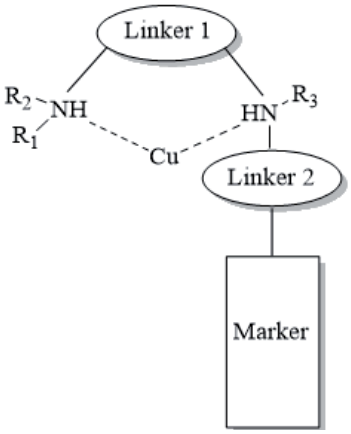


Figure 2. Schematic representation of the copper complexes for amyloid plaques and tau PET imaging

toxicity caused by loss of the metal contained in the synthetic molecule.

The use of PET to image amyloid plaques, combined with tau protein imaging using this medical imaging technique, may further improve the specificity of the diagnosis of AD. Regarding tau protein imaging, it has recently been proposed that the detection of tau by PET imaging is predictive of cognitive decline in AD in domain-specific brain areas, providing important insights into the interaction between tau burden and neurodegeneration, which is critical for the development of new prognostic markers that will help improve the design of therapeutic trials [28,29].

Using the same rationale for designing the ligands for synthesizing copper complexes for PET imaging of amyloid plaques, other new ligands will be designed to develop copper complexes for PET tau imaging (Fig. 2).

Conclusions

In this work, we use predictive models to design and synthesize a novel copper complex that is able to detect amyloid plaques and shows low toxicity to neuronal cells. In the future, this molecule could be used for the development of radiotracers that can be used in PET to detect amyloid plaques and contribute to the diagnosis of Alzheimer's disease. Based on these results, we also propose to develop a new copper complex for amyloid and tau PET imaging.

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