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NMR Investigations of Amyloid-β Peptide Interactions with Propofol at Clinically Relevant Concentrations with and without Aqueous Halothane Solution

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Abstract. Oligomerization of amyloid-β peptide (Aβ) is an important stage in Alzheimer's disease. Recently, it has been shown that in an experimental model, smaller sized anesthetics (e.g., isoflurane, desflurane, etc.) induce Aβ oligomerization. Using state-of-the-art solution nuclear magnetic resonance, spectroscopic studies on Aβ interaction with propofol indicated that propofol does not interact with the G29, A30, and I31 residues of Aβ peptide at a clinically relevant concentration (0.083 mM), and no Aβ oligomerization was observed after 69 days. However, Aβ oligomerization was observed when treated with propofol (clinically relevant concentration) co-administered with aqueous halothane solution. Furthermore, dose dependence studies at various propofol concentrations (0.32 mM, 2.07 mM, and 53.4 mM) indicate the effect of propofol concentration on Aβ oligomerization revealing the hydrophobic nature of interactions between propofol with these critical residues (G29, A30, and I31). These experimental findings reaffirm that smaller molecular sized anesthetics (e.g., halothane) do play a leading role in Aβ oligomerization.

Keywords: Aβ oligomerization, amyloid-β peptide, clinically relevant concentration, propofol, NMR, halothane

INTRODUCTION

Oligomeric amyloid-β peptides (Aβ) are believed to play an important role in Alzheimer's disease (AD), a major health concern for the elderly population worldwide. Any chemical agent promoting oligomeric Aβ formation could be considered as a risk factor for AD. Hence, studies on the influence of anesthetics on Aβ are important and timely as biophysical studies have shown that smaller sized inhaled anesthetics promote Aβ oligomerization [1–4]. A report from the Journal of the American Medical Association indicated: "Some of the commonly used inhaled anesthetics may cause brain damage that accelerate the onset of Alzheimer's disease" [5].

Animal model studies have reported deleterious impact of isoflurane (twice a week, for 3 months) on behavior, survival, neuronal cell death, and processing of proteins involved in neurodegeneration on AβPPswe transgenic mice, 7 to 10 months of age but does not affect normal WT mice [6].

We have reported earlier, using state-of-the-art nuclear magnetic resonance (NMR) techniques, on the molecular interactions of Aβ with isoflurane, desflurane, thiopental, and thiopental co-administered with

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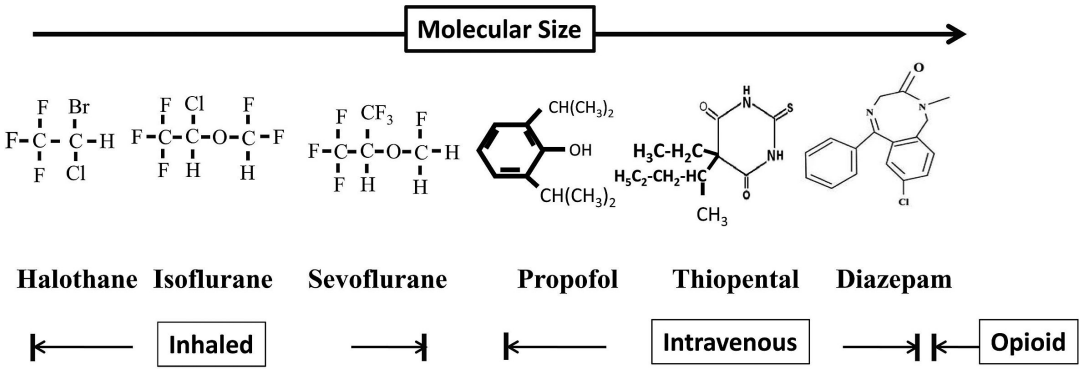

Fig. 1. Molecular structure of anesthetics based on molecular volume (Å^3). The molecular structure of propofol is in the intermediate range between smaller (inhaled) and larger sized (intravenous) anesthetics. The calculated molecular volume of halothane, isoflurane, and propofol are 90 Å^3, 140 Å^3, and 191 Å^3 [32,33] respectively.

halothane, diazepam, and diazepam co-administered with halothane [2–4,7]. Our studies demonstrated that the inhaled anesthetics isoflurane and desflurane, at clinically relevant concentrations, interact with specific amino acid residues (G29, A30, and I31) of the Aβ peptide located in the helix-loop-helix region, and induce Aβ oligomerization [2,3]. It is important to note that bulkier sized thiopental [4], as well as diazepam [1], do not induce Aβ oligomerization as these anesthetics could not access the specific region containing three crucial amino acid residues (G29, A30, and I31) of the Aβ peptide. However, when these bulkier sized anesthetics were co-administered with halothane, Aβ oligomerization was observed, leading to the conclusion that smaller sized halothane induced Aβ oligomerization. The presence of bulkier sized intravenous anesthetics, thiopental or diazepam, does not block smaller sized halothane from accessing the cavity containing three critical amino acid residues (G29, A30, and I31) and subsequently, induction of Aβ oligomerization was observed.

Propofol, a widely used, intermediate sized intravenous anesthetic, oligomerizes Aβ peptide at higher concentrations in experimental models (Fig. 1). Hence, it is important to investigate whether the popularly used propofol, at clinically relevant concentrations, has any role in Aβ oligomerization. In continuance of such systematic propofol-Aβ interactions study, it would be equally important to test whether propofol (at a clinically relevant concentration) co-administered with smaller sized anesthetics (e.g., halothane) promotes any Aβ oligomerization. It is also crucial to investigate concentration dependent effect of propofol on Aβ peptide.

We would like to accomplish three objectives:

1. To investigate if the widely used intermediate sized anesthetic, propofol (at clinically relevant concentrations) singularly, or co-administered with halothane, causes Aβ oligomerization.
2. To test our hypothesis that the smaller molecular sized anesthetic plays an important role in Aβ oligomerization.
3. To investigate the dose dependence of propofol on Aβ.

To address the above-mentioned research objectives, we have used solution-state NMR spectroscopy to investigate Aβ peptide interaction with (i) propofol (at a clinically relevant concentration), (ii) propofol (at a clinically relevant concentration) co-administered with an aqueous halothane solution in time-dependent studies, and (iii) various propofol concentrations.

MATERIALS AND METHODS

Sample preparation

Deuterated d_{25}-SDS (Cambridge Isotope Laboratories), uniformly ^{15}N-labeled Aβ_{40} peptide (Recombinant Peptide Technologies, Atlanta, GA, USA), propofol (Aldrich), halothane (Sigma) have been used in this study.

Uniformly ^{15}N-labeled Aβ peptide was added to d_{25}-SDS solution and gently mixed. The pH of the Aβ peptide solution was adjusted to 7.2 before addition of propofol. The final volumes of the Aβ peptide solution for NMR studies were 500 μl (for control experiment), and 505 μl for Aβ+ propofol (at clinically relevant concentration). The final Aβ and propofol concentra-

tions in Aβ-propofol solution were 0.22 and 0.083 mM respectively.

Preparation details for aqueous halothane solution are provided in our previous work [7]. Aqueous halothane solution (75 μl) was then added to the freshly prepared 505 μl (Aβ+ propofol) solution. The final Aβ and propofol concentrations were 0.26 mM and 0.0725 mM in a total volume of 580 μl.

For propofol dose dependence studies, fresh propofol stock solution was prepared and 3 μl and 20 μl stock solution was added separately to two NMR tubes each containing 500 μl Aβ peptide solution. For interaction studies at high propofol concentration, 5 μl neat propofol was added directly to the 500 μl Aβ solution in an NMR tube. For dose dependence studies, the three propofol concentrations (0.32 mM, 2.07 mM, and 53.4 mM) were used separately for NMR studies.

NMR spectroscopy

All NMR experiments were carried out at 30°C on a Bruker Avance III spectrometer equipped with cryogenic triple-resonance probes, operating at field strength of 500 MHz. Temperature calibration was performed using 100% d_4-methanol sample [8]. One dimensional ^{1}H NMR and 2D [^{15}N,^{1}H] heteronuclear single quantum coherence (HSQC) spectra were measured using TOPSPIN 2.1 for ^{15}N-labeled Aβ [9]. To study Aβ oligomerization four sets of NMR experiments were designed: (i) Aβ peptide only (control), (ii) Aβ+ propofol (at clinically relevant concentration), (iii) Aβ+ propofol + halothane, and (iv) Aβ+ propofol at three different concentrations.

For Aβ alone or Aβ+ propofol (at a clinically relevant concentration), one dimensional ^{1}H spectra were recorded, followed by 2D [^{15}N,^{1}H] HSQC spectra at 7 different time points (up to a total of 69 days).

For Aβ+ propofol (at a clinically relevant concentration) co-administered with halothane aqueous solution, one dimensional ^{1}H spectra were recorded, followed by 2D [^{15}N,^{1}H] HSQC spectra at 5 different time points (up to a total of 8 days).

Detailed procedure for NMR studies is described in our previous work and will not be elaborated here. NMR data were processed and analyzed using nmrPipe [10], PIPP [11], and SPARKY [12] on an Octane2 silicon graphics workstation. Assignments of the amide peaks of Aβ were taken from our earlier work [1, 2,4] performed in a similar environment.

RESULTS

In the present study, Aβ interactions with intermediate sized anesthetics, propofol (clinically relevant concentration), singularly and co-administered with halothane, have been investigated using solution-state NMR spectroscopy. NMR studies were also performed at various propofol concentrations for dose dependence studies on Aβ oligomerization.

HSQC experiments for both Aβ (only) and Aβ+ propofol (0.083 mM) were collected at 7 different times points spanning total 69 days and no Aβ oligomerization was observed after 69 days in both experimental conditions (Aβ alone or Aβ+ propofol (0.083 mM) [13]. Figure 2A shows the overlay of two [^{15}N,^{1}H] HSQC spectra of Aβ only (blue) and Aβ+ propofol (red). It is important to mention that intensities and/or chemical shift of the ^{1}H peaks of propofol remain unaltered.

Figure 2B presents the overlay of [^{15}N,^{1}H] HSQC spectra of Aβ peptide (blue) and Aβ+ propofol (0.072 mM) co-administered with 75 μl halothane solution (red). The critical residues G29, A30, and I31 show chemical shift changes due to halothane. Aβ oligomerization [13] was observed on 8th day. The amide peak intensities of Aβ peptide are also reduced and there is the appearance of additional peaks indicating Aβ oligomerization [4,5].

Figure 2C presents the overlay of [^{15}N,^{1}H] HSQC spectra of Aβ peptide (blue), Aβ peptide + propofol (53.4 mM) after 2 hours (green color), and Aβ peptide + propofol (53.4 mM) after 6 days (red color). The critical residues G29, A30, and I31 show chemical shift changes due to interaction with propofol. After 6 days, Aβ oligomerization was found in the presence of 53.4 mM propofol concentration. It is important to mention that Aβ oligomerization was observed in all three cases at 0.32 mM, 2.07 mM, and 53.4 mM propofol concentrations. Aβ oligomerization was more severe in the presence of 53.4 mM propofol concentration compared to the other two experimental conditions (Aβ + 0.32 mM propofol and Aβ + 2.07 mM propofol). The severe nature of Aβ oligomerization is reflected by the drastic reduction of amide peak intensities of Aβ peptide and the appearance of broad peaks, indicated by arrows. In the presence of 0.32 mM propofol, Aβ oligomerization is less compared to 2.07 mM propofol (data not shown).

DISCUSSION

Our previous studies with larger sized intravenous anesthetic (e.g., thiopental and diazepam) indicated that these two anesthetics, due to bulkier size, could not

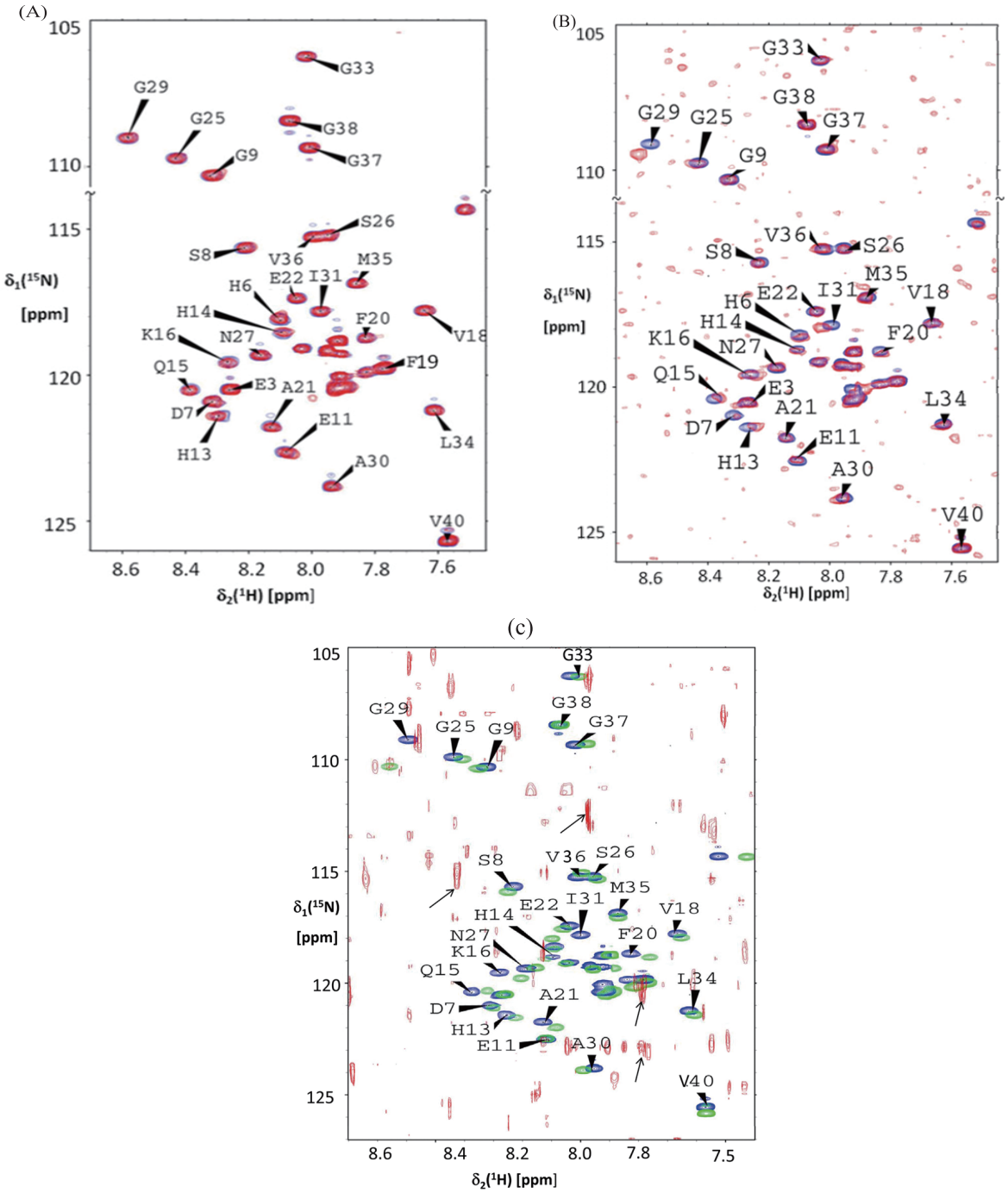

Fig. 2. A) 2D [^{15}N,^{1}H] HSQC spectra of Aβ peptide in the presence of 0.0832 mM propofol solution. The HSQC spectrum (control), Aβ without propofol, is shown in blue, and the HSQC spectra in the presence of propofol on 69 days are shown in red, respectively. Backbone amides of residues G29, A30 and I31 do not show any change in chemical shift upon addition of propofol. B) 2D [^{15}N,^{1}H] HSQC spectra of Aβ peptide + propofol (0.072 mM) co-administered with 75 μl halothane solution. The HSQC spectrum of Aβ without propofol is shown in blue, and the HSQC spectra in the presence of propofol co-administered with halothane is shown in red. Backbone amides of residues G29, A30 and I31 show changes in their chemical shifts on addition of halothane, indicating oligomerization of Aβ in the sample. C) 2D [^{15}N,^{1}H] HSQC spectra of Aβ peptide in the 53.4 mM propofol solution. The HSQC spectrum (control), Aβ without propofol is shown in blue, the HSQC spectra in the presence of propofol (53.4 mM) are shown in green, and HSQC spectra in the presence of propofol after 6 days are shown in red. Backbone amides of residues G29, A30 and I31 show chemical shift change in the presence of propofol (53.4 mM). This confirms the presence of oligomeric amyloid beta states, which are marked by arrows.

access the specific helix-loop-helix region of Aβ peptide containing the critical amino acid residues (G29, A30, and I31). Hence, there was no Aβ oligomerization induced by these intravenous anesthetics at very high, as well as low, concentration [1,4]. On the contrary, smaller sized inhaled anesthetics (isoflurane and desflurane) at clinically relevant concentrations induce chemical shift of those critical resides, and subsequently Aβ oligomerization was observed [2,3]. Propofol is an intermediate sized (191 $Å^3$) intravenous anesthetic and its molecular size lies in between inhaled and other bulkier sized intravenous anesthetics (e.g., benzodiazepines or thiopental etc).

Concentration effect of propofol on Aβ oligomerization

Propofol at a higher concentration (64 mM), induces Aβ oligomerization [4]. The propofol concentration used in our previous studies was much higher than clinically relevant concentrations. Hence, our present study design was made keeping in mind Aβ peptide interactions with propofol concentration at a clinically relevant concentration. In order to investigate the effect of dose dependence of propofol on Aβ peptide, NMR experiments were also performed with three propofol concentrations (0.32 mM, 2.07 mM and 53.4 mM) respectively. At very high concentrations, interactions of propofol with crucial residues are profound and, subsequently, Aβ oligomerization is severe, whereas, in 0.32 mM and 2.07 mM concentrations, relatively moderate Aβ oligomerization is found. Between two propofol concentrations, Aβ oligomerization is relatively lower in 0.32 mM, compared to 2.07 mM, propofol concentrations.

Aβ peptide, without propofol (data not shown) and with propofol at clinically relevant concentrations (Fig. 2A) are stable and no oligomerization was observed after 69 days in both cases. However, at high concentrations propofol could reach the cavity containing three crucial residues which indicates that the molecular volume of propofol is the same range as the cavity size. Furthermore, the dose dependence studies with various concentrations of propofol (0.32 mM, 2.07 mM, and 53.4 mM) with Aβ peptide establish the hydrophobic nature of the interaction between propofol and these critical residues. Other studies in different system have also reported a hydrophobic nature of interactions between anesthetics and protein [14].

Another important observation from these NMR studies is that bulkier sized anesthetics, e.g., thiopental [4] and diazepam [1], when co-administered with halothane, could not prevent access of smaller sized halothane molecules to the helix-loop-helix region containing three crucial residues (G29, A30, and I31). A similar observation is found when propofol (at a clinically relevant concentration) is co-administered with halothane, and Aβ oligomerization was inititated.

The schematic diagram for the Aβ peptide and propofol at a clinically relevant concentration is presented in Fig. 3. The interactions study between Aβ peptide and anesthetics are supported by the topological coexistence of both Aβ and anesthetics in the extracellular domain [1–4]. Aβ peptide is produced by subsequent cleavage of β- and γ-secretase on amyloid-β protein precursor (AβPP) protein in the normal biological process of aging [4]. Hence, it is likely that the aged brain has redundant Aβ peptide. Therefore, the possibility of anesthetic interactions with Aβ peptide is much higher in an aged population. This schematic model based on biophysical studies provides vital information regarding the molecular pathway for Aβ oligomerization by anesthetics, as well as providing guidance for designing future inhaled anesthetics with appropriate molecular size.

Clinical importance of this biophysical research

These findings, despite evidence in clinical setting yet, have a potential clinical relevance for caregivers. Uncontrolled Aβ amyloid aggregation and deposition is the characteristic hallmark of AD, and the major factor in causing neurodegeneration and dementia [15–17]. Certain general anesthetics commonly administered in clinical practice have demonstrated the capability of promoting Aβ aggregation in biophysical studies [2–4,7].

Aβ is naturally contained in the brain, with increased levels in the elderly [18], but increased concentrations of Aβ are also pathologically present in the brain of subjects with brain damage, as well as patients admitted to intensive care unit (ICU) for brain injuries and trauma [19–22], or neurodegenerative diseases (i.e., AD or Down syndrome). Higher amounts of Aβ are available to interact with anesthetics during general anesthesia or prolonged sedation in ICU: therefore, the administration of general anesthetics which affect the rate at which Aβ bind together may promote and accelerate oligomerization.

The finding that propofol, at clinical concentrations, does not promote Aβ oligomerization has a relevant importance for clinical practice, since it is rapidly becom-

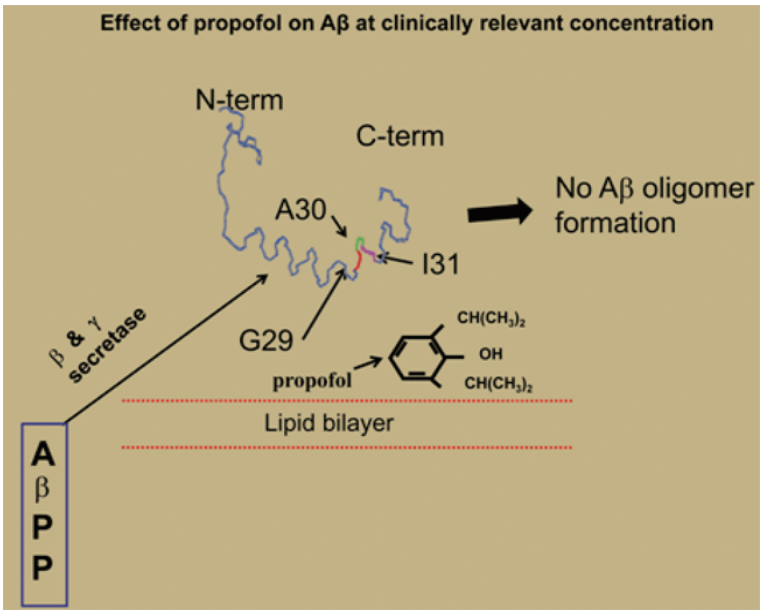

Fig. 3. Schematic diagram for interaction studies of Aβ peptide with propofol. At clinically relevant concentration of intermediate sized anesthetics, propofol could not reach the critical residues G29, A30 and I31 located in the helix-loop-helix region: these critical residues are accessible for interaction for halothane and promote Aβ oligomerization. The figure is adapted and revised from our earlier works [1,2,4].

ing one of the most popular agents for general anesthesia, sedation, and invasive procedures [23]. Because of propofol's remarkable safety profile, pharmacokinetic characteristics that allow for rapid onset and offset of drug effects, and fast elimination from the body, this hypnotic agent is often administered to the elderly, in context of a continuously growing population of older patients who require an increasing number of anesthetics, and sedation [24,25]. For induction of general anesthesia, propofol is a suitable agent in patients with neurodegenerative disease [26]. In ICU, propofol is the most commonly used agent for sedation, mechanical ventilation, and diagnostic and therapeutic procedure facilitation [27] in the brain-injured patient [28], and, in general, in neuro-intensive care [29]. To some extent, propofol is also used with elderly patients to provide sedation during regional anesthesia that may mitigate prolonged surgery [30].

Although propofol is considered as safe from the point of uncontrolled Aβ aggregation, unfortunately, it also has serious adverse effects that must be carefully considered [29]. In addition, caregivers should be aware that, in spite of its positive effects and remarkable safety profile as mentioned above, it has been associated with the "propofol syndrome", a potentially fatal complication essentially characterized by metabolic acidosis, rhabdomyolysis, arrhythmias, myocardial and renal failure, hepatomegaly, and death [31]. There is a lack of clinical studies that would support the use of any one anesthetic agent (i.e., propofol) over others. Therefore, it is too early to recommend any specific agent for clinical use, and further study is warranted to unravel relevant mechanisms, and appreciate the potential clinical relevance of our experimental findings.

CONCLUSIONS

NMR studies for Abeta-propofol interactions provide the binding pocket compared to other biophysical technique [34].

Smaller sized inhaled anesthetics do promote Aβ oligomerization based on biophysical and animal model studies. Our NMR studies provide guidance for designing new inhaled anesthetics which should satisfy all clinical characteristic of inhaled anesthetic molecules, as well as having molecular volume should be close to 191 Å^3. Once this type of inhaled anesthetic, with appropriate molecular size, is designed and synthesized, this NMR experimental protocol will provide an important platform to test Aβ oligomerization propensity.

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