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Inhibition of Aβ production by NF-κB inhibitors

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Abstract

The transcription factor nuclear factor κB (NF-κB) is widely expressed in the nervous system and increased NF-κB immunoreactivity has been observed in Alzheimer's disease (AD) brains in the nuclei of neurons within the vicinity of diffuse β-amyloid plaques. β-Amyloid (Aβ) peptides are the main constituent of senile plaques and are known to stimulate NF-κB activity. In the present study, we investigated the effect of various NF-κB inhibitors on the production of $A\beta_{1–40}$, $A\beta_{1–42}$, secreted APP (sAPPβ and sAPPα) and APP C-terminal fragments (APP-CTF) using CHO cells overexpressing the β-amyloid precursor protein (APP). Our data show that NF-κB inhibitors decrease both $A\beta_{1–40}$ and $A\beta_{1–42}$ production. In addition, we show that some NF-κB inhibitors decrease sAPPβ and APP-CTFβ suggesting that they reduce the β-secretase cleavage of APP. Altogether our data suggest that NF-κB inhibitors may be of therapeutic importance for the treatment of AD pathology not only by blocking inflammatory processes but also by directly inhibiting the production of Aβ peptides.

Keywords: Alzheimer; APP processing; NF-κB; Amyloid

The brains of Alzheimer's disease (AD) patients are characterized by the accumulation of a 38–43-amino acids peptide termed amyloid β-peptide or Aβ [16]. This peptide is the major component of senile plaques found in AD and is proposed as a key player in the pathobiology of AD since all familial forms of the disease are associated with an increased Aβ accumulation. Aβ is a proteolytic fragment of the large amyloid precursor protein (APP) [6]. It is cleaved by β-secretase generating secreted APPβ (sAPPβ) and the carboxyl terminal intracellular fragment (APP-CTFβ) which is further cleaved by γ-secretase to produce Aβ peptides [17], whereas the α-secretase cleavage of APP takes place within the Aβ sequence precluding Aβ production, leading to the secretion of sAPPα and the accumulation of membrane-coupled APP CTF-α. The γ-secretase cleavage is not precise and leads to the production of a series of Aβ peptides of 38–43 amino acids. $A\beta_{1–42}$ is more fibrillogenic than shorter Aβ peptides but a higher proportion of Aβ is produced as $A\beta_{1–40}$ compared to $A\beta_{1–42}$. Elevated $A\beta_{1–42}$ concentrations are thought to drive the formation of insoluble fibrils resulting in the deposition of Aβ as amyloid plaques. For this reason $A\beta_{1–42}$ is considered to have a causative role in the etiology of AD and therapies particularly targeting $A\beta_{1–42}$ are expected to have the greatest impact on β-amyloid pathology.

The transcription factor NF-κB is widely expressed in the nervous system and particularly in synaptic terminals. In non-stimulated cells, NF-κB is sequestered in the cytoplasm by inhibitory units called IκB proteins. Stimulation of cells by various inducers causes IκB phosphorylation and its subsequent degradation by the proteasome [21]. Liberated NF-κB is transported in the nucleus, where it induces transcription of target genes, including IκB as an autoregulatory loop [21].

Studies of postmortem brain tissue from patients with AD have revealed increased NF-κB immunoreactivity in neurons and astrocytes in the immediate vicinity of β-amyloid plaques [7,19]. Many genes newly induced in AD are under immediate-early transcriptional control of NF-κB [11] also suggesting that NF-κB pathway is activated in AD brains. Other studies have shown that Aβ peptides can activate NF-κB in primary neurons and astrocytes [2] suggesting a molecular mechanism by which Aβ may act during AD pathogenesis. Recently, it has been reported that indomethacin (a non steroidal anti-inflammatory drug) can lead to a reduction in the level of Aβ peptides and NF-κB in the brains of a transgenic mouse model of AD (Tg2576) whereas another anti-inflammatory compound, nimesulide had no effect on either Aβ peptides or NF-κB [18] suggesting that

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NF-κB activation blockade could reduce the amyloid pathology in Tg2576 mice. Since Aβ itself is known to induce NF-κB, the reduced NF-κB activity observed in Tg2576 mice treated with indomethacin could alternatively be secondary to the reduction of Aβ accumulation observed. We therefore investigated the effect of various NF-κB inhibitors on the production of Aβ peptides using Chinese Hamster Ovary cells stably transfected with wildtype APP 751 (7W CHO), overproducing human Aβ. The effect of NF-κB inhibitors was also tested on the accumulation of APP C-terminal fragments (APP-CTF) in order to determine whether NF-κB inhibitors could impact γ-secretase activity. Additionally, we investigated the effect of NF-κB inhibitors on the secretion of sAPPα and sAPPβ to determine a possible effect of NF-κB inhibitors on α-secretase or β-secretase activities.

For this study, we tested the effect of different NF-κB inhibitors (NF-κB SN50, parthenolide, hypoestoxide, capsaicin, andrographolide, Caffeic Acid Phenethyl Ester (CAPE), artemisinin, celastrol, 6-amino-4-(4-phenoxyphenyl-ethylamino)quinazoline (quinazoline), isohelenin, kamebakaurin) on the production of $A\beta_{1–40}$ and $A\beta_{1–42}$ peptides by CHO cells overexpressing APP. NF-κB SN50 is a selective, cell permeable NF-κB inhibitor peptide which contains the nuclear localization sequence (NLS) of the transcription factor NF-κB p50 linked to the hydrophobic region (h-region) of the signal peptide of Kaposi fibroblast growth factor (K-FGF). The N-terminal K-FGF h-region confers cell-permeability, while the NLS inhibits translocation of the NF-κB active complex into the nucleus [14]. Parthenolide and isohelenin are two sequiterpone lactone known to block NF-κB activation [3]. Andrographolide is a bicyclic diterpenoid lactone, which inhibits NF-κB binding to DNA [8]. CAPE is an active component of propolis from honeybee hives that prevents the translocation of the p65 subunit of NF-κB to the nucleus [15]. Artemisinin (a potent anti-malarial sesquiterpene) and celastrol (sesquiterpene ester) have been shown to block NF-κB activation [1,10]. Kamebakaurin is a kaurane diterpene, which prevents the DNA binding activity of activated NF-κB but does not block its nuclear translocation [13]. Quinazoline is a low molecular weight inhibitor of NF-κB transcriptional activation [20].

Briefly, 7W CHO cells were maintained in DMEM (ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 1× Penicillin–Streptomycin Fungizone mixture (Cambrex, Rockland, ME, USA) and 0.3% Geneticin (Invitrogen, Carlsbad, CA, USA). Cells were plated on 96 well-culture plates at a density of 5×10^4 cells per well in 200 μL of culture medium. All the inhibitors used in this study were purchased from EMD Biosciences Inc. (San Diego, CA, USA). The concentration of inhibitors used to achieve NF-κB inhibition were chosen according to referenced publications. For each inhibitor, experiments were performed in quadruplicate and $A\beta_{1–40}$ and $A\beta_{1–42}$ levels were evaluated following 18 h of incubation using ELISA kits (Invitrogen Biosource Inc., Carlsbad, CA, USA) according to the recommendations of the manufacturer. For the measurement of $A\beta_{1–40}$, samples were diluted 20-fold with the sample diluent provided in the kits whereas for $A\beta_{1–42}$ determination, samples were diluted only two-fold. Results were expressed as the percentage of $A\beta_{1–40}$ or $A\beta_{1–42}$ measured in control samples. No direct toxicity (monitored by lactate-dehydrogenase release in the culture medium) was observed for the doses of NF-κB inhibitors tested (data not shown). Interestingly, the different NF-κB inhibitors tested appear to diminish Aβ production with different potency and show a differential effect towards $A\beta_{1–40}$ and $A\beta_{1–42}$ production (Fig. 1A). For instance, NF-κB SN50 at 20 μM inhibits $A\beta_{1–42}$ (∼40% inhibition) more potently than $A\beta_{1–40}$ (∼9%). Similarly, hypoestoxide displays more potency towards $A\beta_{1–42}$ than $A\beta_{1–40}$ inhibition. Quinazoline, artemisinin (at 1 μM) and isohelenin (at 5 μM) inhibit $A\beta_{1–40}$ secretion more potently than $A\beta_{1–42}$ whereas the other compounds tested display analogous effects on $A\beta_{1–40}$ and $A\beta_{1–42}$ production.

NF-κB activation can be blocked indirectly by inhibiting I kappa B kinase (IKK-2). We therefore tested a cell-permeable ureidocarboxamido thiophene compound that acts as a potent inhibitor of IKK-2 ([5-(*p*-Fluorophenyl)-2-ureido]thiophene-3-carboxamide or IKK-2 inhibitor IV). IKK-2 inhibitor IV also appears to significantly inhibit both $A\beta_{1–40}$ and $A\beta_{1–42}$ production (Fig. 1A). These data support the concept that the secretion and/or production of $A\beta_{1–40}$ and $A\beta_{1–42}$ is NF-κB dependent.

In order to verify that NF-κB inhibitors were impacting the secretion of Aβ, we measured the intracellular level of $A\beta_{1–40}$. Briefly, 7W CHO were treated with different NF-κB inhibitors (Fig. 1B). Following 18 h of incubation, cells were washed with PBS and lyzed with 100 μL of ice-cold M-PER® Reagent (Pierce, IL, USA) containing 1 mM phenylmethanesulfonyl fluoride and 1 mM sodium orthovanadate. Cellular lysates were diluted five-fold with the standard diluent provided in the $A\beta_{1–40}$ ELISA kit (Biosource, CA) before intracellular $A\beta_{1–40}$ quantification. Data show that intracellular $A\beta_{1–40}$ represent only a small fraction of the $A\beta_{1–40}$ produced by the cells (approximately 1.5% of the amount of $A\beta_{1–40}$ secreted in the culture medium). Interestingly, among the NF-κB inhibitors tested, only CAPE, celastrol and kamebakaurin were able to significantly lower intracellular $A\beta_{1–40}$, but no intracellular accumulation of Aβ was observed with any of the compounds suggesting that the secretion of Aβ is not impacted by NF-κB inhibition (Fig. 1B).

In order to verify that NF-κB inhibition was achieved, we measured the production of prostaglandin E_2 (PGE_2), a main product of cyclooxygenase-2 (whose expression is regulated by NF-κB) employing the same culture paradigm used to measure Aβ production. This was necessarily an indirect way of assessing NF-κB inhibition as the inhibitors are purported to achieve their effects by different mechanisms (some inhibit nuclear translocation of NF-κB, while others inhibit its binding to DNA). We analyzed conditioned cell culture media for PGE_2 (a main metabolite of cyclooxygenase-2, expression of which is NF-κB regulated), using a commercially available ELISA according to the strict recommendations of the manufacturer (Cayman Chemical, Ann Arbor, MI, USA), following treatment of 7W CHO cells with various NF-κB inhibitors for 18 h. Our results (Fig. 2) demonstrate a differential inhibition of 7W CHO cell PGE_2 production showing that the NF-κB inhibitors tested inhibit NF-κB activity with different potencies [4]. Interestingly, we found a correlation between the amount of PGE_2 inhibition and the