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CURCUMINOIDS ENHANCE MEMORY IN AN AMYLOID-INFUSED RAT MODEL OF ALZHEIMER'S DISEASE

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Abstract—Alzheimer's disease (AD) is a neurodegenerative disease. There are a limited number of therapeutic options available for the treatment of AD. Curcuminoids (a mixture of bisdemethoxycurcumin, demethoxycurcumin and curcumin) is the main chemical constituent found in turmeric, a well known curry spice, having potential in the treatment of AD. The objective of this study was to investigate the effects of curcuminoid mixture and individual constituents on spatial learning and memory in an amyloid-beta (Aβ) peptide-infused rat model of AD and on the expression of PSD-95, synaptophysin and camkIV. Curcuminoid mixture showed a memory-enhancing effect in rats displaying AD-like neuronal loss only at 30 mg/kg, whereas individual components were effective at 3–30 mg/kg. A shorter duration treatment with test compounds showed that the curcuminoid mixture and bisdemethoxycurcumin increased PSD-95 expression in the hippocampus at 3–30 mg/kg, with maximum effect at a lower dose (3 mg/kg) with respective values of 470.5 and 587.9%. However, after a longer duration treatment, two other compounds (demethoxycurcumin and curcumin) also increased PSD-95 to 331.7 and 226.2% respectively at 30 mg/kg. When studied for their effect on synaptophysin in the hippocampus after the longer duration treatment, the curcuminoid mixture and all three individual constituents increased synaptophysin expression. Of these, demethoxycurcumin was the most effective showing a 350.1% increase ($P<0.01$) at 30 mg/kg compared to the neurotoxin group. When studied for their effect on camkIV expression after longer treatment in the hippocampus, only demethoxycurcumin at 30 mg/kg increased levels to 421.2%. These compounds salvaged PSD-95, synaptophysin and camkIV expression levels in the hippocampus in the rat AD model, which suggests multiple target sites with the potential of curcuminoids in spatial memory enhancing and disease modifying in AD.

Key words: curcumin, bisdemethoxycurcumin, demethoxycurcumin, genes, memory, hippocampus.

Alzheimer's disease (AD) is one of the most common forms of dementia, accounting for 50–60% of dementia cases (Blennow et al., 2006) and is associated with the deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles (composed of paired helical filaments) in the brain of AD patients (Selkoe, 2001). Amyloid plaques are principally composed of 40–42 residue long peptides (Aβ peptide). Amyloid peptide (Aβ) is established as a neurotoxic, having a devastating effect on nerve cells (Selkoe, 2001; Roncarati et al., 2002) and is responsible for memory deficits predominantly through the disruption of synaptic functions (Nimmrich and Ebert, 2009).

Synaptic plasticity is maintained through various key players at the pre-synaptic and post-synaptic junctions. Post-synaptic density protein (PSD-95) is expressed at the post-synaptic membrane where it has been involved in the coupling of the NMDA receptors and K^+ channels and forms a functional complex in the post-synaptic membrane (Kim et al., 1996). PSD-95, thus plays a key role in the process of learning and it is known that PSD-95 mutant mouse shows memory deficit (Migaud et al., 1998). Synaptophysin is a synaptic vesicle membrane protein, expressed throughout the brain and is responsible for the activity-dependent synapse formation (Tarsa and Goda, 2002). How expression of these molecules changes in AD and contributes towards the mechanism of pathogenesis is not clear. Similarly, how potential therapeutic candidates modulate their pattern to show a beneficial effect in AD subjects, remains to be elucidated.

There is substantial evidence supporting the notion that compounds derived from the medicinal plants have played a leading role in many diseases (Gilani and Rahman, 2005; Corson and Crews, 2007; Schmidt et al., 2007), particularly in dementia (Le Bars et al., 1997; Akhondzadeh et al., 2003; Choudhary et al., 2005).

Turmeric is a popular spice considered useful in AD and a common belief is that the effectiveness of turmeric in many diseases, including AD, is due to the presence of its main constituent curcumin (Cole et al., 2007; Aggarwal and Sung, 2009). Curcuminoid, a key constituent of turmeric, is a mixture of three chemical constituents (bisdemethoxycurcumin 3–5%, demethoxycurcumin 15–20% and curcumin 75–80%) but the common approach in various studies has been to use curcumin and curcuminoid as the same entity probably because curcumin constitutes the major part, and it is the most extensively studied constituent. However, in our recent study we observed that, though curcumin was equally effective in scopolamine-induced amnesia it showed distinctly less effectiveness in acetylcholinesterase (AChE) inhibitory activity when compared to the parent curcuminoid mixture or two other compounds (Ahmed and Gilani, 2009). As AChE inhibition represents

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Abbreviations: Aβ, amyloid peptide; AChE, acetylcholinesterase; AD, Alzheimer's disease; LTP, long term potentiation; NMDA, *N*-methyl-D-aspartate; PSD-95, post synaptic density protein-95; RT-PCR, reverse transcription polymerase chain reaction.

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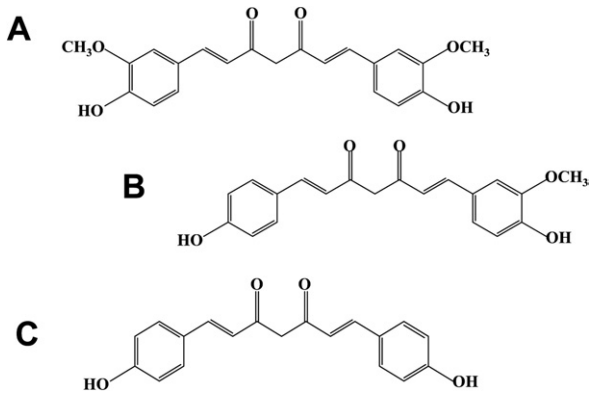

Fig. 1. Chemical structure of curcumin (A), demethoxycurcumin (B) and bisdemethoxycurcumin (C).

one out of multiple mechanisms useful in memory deficits in AD, it is likely that curcumin would show more effectiveness in its memory enhancing effect in other models.

The prime question that we put forward was whether these properties of the curcuminoid mixture are primarily due to the presence of curcumin, and how the other two constituents contribute in the potential significance of curcuminoid mixture in AD. Consequently, we compared the effect of curcuminoid mixture and individual constituents on spatial memory deficits in rats showing AD-like hippocampal neuronal loss and on the expression of genes involved in synaptic plasticity.

EXPERIMENTAL PROCEDURES

Drugs and chemicals

Amyloid beta peptide (1–40) and ibotenic acids were purchased from the Sigma Chemical Company, St. Louis, MO, USA. Curcuminoids (greater than 95% purity; having bisdemethoxycurcumin 4.15%, demethoxycurcumin 16.53% and curcumin 79.52%) and its individual components, bisdemethoxycurcumin (78% purity), demethoxycurcumin (98% purity) and curcumin (98.35% purity) were generous gifts from the Sabinsa Group of Companies, 70 Ethel Road West, Unit 6, Piscataway, NJ 08854, USA. The purity of the curcuminoids and individual components was established by the Sami Laboratories Ltd., Banglore, India (part of Sabinsa Group of Companies) through HPLC (Ahmed and Gilani, 2009). Ketamine, diazepam and buprenorphine were purchased from the Aga Khan Hospital, Karachi.

Animals

The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996) and the protocol was approved by the Ethical Committee for Research on Animals (ECRA), Aga Khan University. Male, Sprague–Dawley rats (180–250 g and 14–18 weeks of age) were bred and housed in the animal house of the Aga Khan University under a controlled environment (23–25 °C). Animals were given tap water *ad libitum* and a standard diet consisting of (g/kg): flour 380, fiber 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium meta bisulfate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

Aβ infused model of AD and study design

Surgery. Amyloid fragment concentration of 7.14 μg/μl was incubated at 37 °C for 5–7 days to form neurotoxic fibrils (Yang et al., 2005). After the incubation period, peptide solution was mixed with an equal volume of ibotenic acid solution (concentration 5 μg/μl). Rats were made to fast for 3–5 h before surgery. Before surgery, the rats were injected with cocktail of drugs to induce anesthesia (ketamine 60 mg/kg, diazepam 5 mg/kg and buprenorphine 0.03 mg/kg). After anesthesia, the head of the animal was fixed in a stereotaxic frame and, with the help of very fine Hamilton syringe, 2 μl of the neurotoxin (mixture of 1 μl amyloid solution plus 1 μl ibotenic acid solution) was injected bilaterally into the hippocampus (coordinates; bregma-4.2, mediolateral 3.0 and dorsoventral 3.6 mm) using atlas (Paxinos and Watson, 2006). Saline was injected bilaterally into the hippocampus of control group animals using the same procedure as explained above. After surgery, the animals were allowed to recover for a day in their cages.

Although it is known that both Aβ(1–40) and (1–42) peptides have neurotoxic effects, in this study Aβ(1–40) was used, as this peptide is able to show fibril formation in rat brain similar to that found in the AD brain as opposed to the fibrils formed by the Aβ(1–42) (Shin et al., 1997). Similarly, when Aβ(1–40) is combined with ibotenic acid, it shows a significant neuronal loss as opposed to the Aβ peptide or ibotenic acid when used alone (Morimoto et al., 1998; Li et al., 2004; Hu et al., 2005; Nakamura et al., 2006). Hence, based on these observations, Aβ(1–40) was combined with ibotenic acid for the intrahippocampal injection to get an AD model where these test compounds could be studied.

Drug treatment. The animals were allowed to recover post-operatively and treatment was started on post-operative day 2 with compounds (Fig. 1 shows chemical structure of compounds and detailed study design is provided in Fig. 2). Compounds were dissolved in saline (with 0.1% Tween 20) for injection and frozen immediately at −20 °C. On the day of treatment these were thawed and injected (not exceeding more than 1 ml per injection in each rat) i.p. in less than 5 min after thawing to prevent degradation of compounds. We studied the effect of compounds for a short duration (5 days i.p. treatment) and a long duration (20 days i.p. treatment) while, in the control group, only saline (equal volume

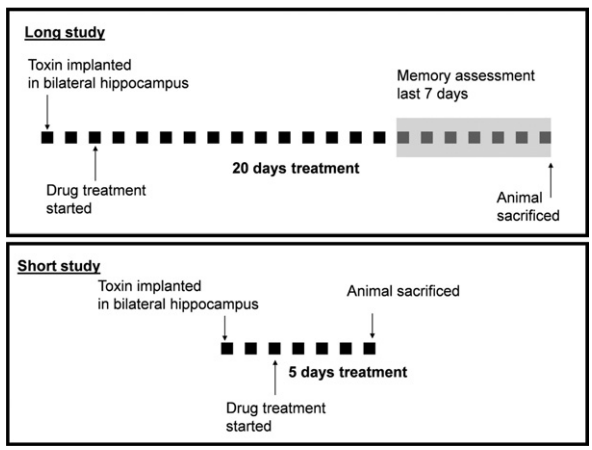

Fig. 2. Diagrammatic representation of the study protocol adopted in Aβ peptide plus ibotenic acid infused rat model of AD: After the surgery, animals were allowed to recover for one day, and treatment with compounds started next day, which continued for either 20 days (long study) or 5 days (short study). Control animals were injected with saline containing 0.1% Tween 20. This solvent (saline with 0.1% Tween 20) was selected after trial and was found to be safe and demonstrated no toxicity in the viscera as well as proving good suspension for i.p. injection. Moreover, we found no lumps formation even after the injection of 30 mg/kg i.p. dose for 20 days. Each small black square represents one day and arrows indicate where the drug treatment started, while the "gray mark" shows where Morris water maze testing was conducted.