Original Article

Promising therapy for Alzheimer's disease targeting angiotensin-converting enzyme and the cyclooxygense-2 isoform

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ABSTRACT: Deposition of β-amyloid in brain is one of the pathological hallmarks of Alzheimer's disease (AD) that is often associated with inflammatory response. Much evidence also points to a link between the renin-angiotensin system, hypertension and dementia. Accordingly, the potential use of anti-inflammatory and antihypertensives might be beneficial agents in AD therapy. In this study, we investigated the possible mechanisms of Celecoxib (cyclooxygenase-2 (COX-2) inhibitor), Perindopril (angiotensin converting enzyme (ACE) inhibitor) and their combination in a lipopolysaccharide (LPS) model of AD. Mice were injected with LPS (0.8 mg/kg, *i.p.*) once then divided into three groups: the first was treated with Celecoxib (30 mg/kg/day, *i.p.*), the second with Perindopril (0.5 mg/kg/day, *i.p.*) and the last group with a combination of both drugs. Learning and memory function were tested using a Y-maze and locomotor activity was assessed using an open-field test. Cerebral specimens were subjected to histopathological studies. Brain tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-1β levels were measured. LPS decreased locomotor activity and percentage of correct choices in the Y-maze test. It also produced a significant increase in the percentage area of vascular angiopathy, area of lamellated plaques, and apoptotic index. These were associated with increased TNF-α and IL-1β. Administration of either Celecoxib or Perindopril partially improved cognitive impairment, decreased inflammatory cytokines and amyloid deposition. Combined therapy of both drugs completely prevented LPS-induced neurodegenerative and cognitive changes. In conclusion, these findings establish a link between COX-2, ACE activity and cognitive impairment in AD and provided a promising strategy for the complete cure of AD.

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1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder marked by progressive loss of memory and cognitive ability. The pathology of AD is characterized by the presence of senile plaques that are deposits of amyloid β protein (Aβ) (*1*), and intracellular neurofibrillary tangles leading to pronounced cell death (*2*). Aβ deposited extracellularly or within the walls of the cerebrovasculature, caused cerebral amyloid angiopathy in 90% of patients with AD (*3*). In addition, the end-stage pathology of AD is also notable for the presence of numerous cellular and molecular markers of an inflammatory response that is often associated with the Aβ deposits. Accordingly, the role of inflammatory mechanisms in the pathogenesis of AD must be taken as a matter of convenience (*4*). In recent years, non-steroidal anti-inflammatory drugs (NSAIDs) have been suggested to be beneficial agents in delaying the onset and possibly reducing the risk of AD (*5-8*), but recommendations for their chronic use are tempered by the well documented risk of gastrointestinal bleeding and ulceration (*9*). Therefore, new classes of NSAIDs have emerged as treatment for AD, including selective inhibitors of cyclooxygenase-2 (COX-2). However, it may be worth noting that a definitive mechanism of action underlying their therapeutic effect remains completely uncertain and still, there is conflicting data regarding the expression of COX-2 in AD (*10,11*). Much epidemiological evidence points to a link between hypertension, risk factors for atherosclerotic vascular disease, and dementia; and in turn, the use of antihypertensive medications has been suggested to reduce the incidence of dementia including Alzheimer's disease, although through an unknown mechanism (*12*). Recent findings indicate that the brain has its own renin-angiotensin system (RAS), which

plays a role in neuronal plasticity as well as in learning and memory (*13*). There is conflicting evidence about the neurobiological links between the renin-angiotensin system and the pathogenesis of Alzheimer's (*14*). It has been reported that angiotensin converting enzyme (ACE) degrades the amyloid β-protein *in vitro*, and is a putative upstream initiator of Alzheimer's disease. This supports the hypothesis that ACE inhibitor (ACEI) treatment might increase Aβ concentrations and could increase Alzheimer's disease risk (*15*). This is in contrast to what has been reported in clinical studies that ACE inhibitors had a beneficial effect on the rate of cognitive decline, thought to be due partly to the presence of Aβ (*16*). Our goal was to investigate the effect of Perindopril, a brain penetrating ACE inhibitor on an Alzheimer's mouse model, to reveal the role of ACE in the pathogenesis of Alzheimer's. We also studied the effect of Celecoxib, a specific cyclooxygenase-2 inhibitor, aiming to explore its mechanism of action. We tested these drugs in terms of cognitive function, amyloidogenesis and inflammatory mediators. Furthermore, we proposed a promising therapeutic strategy based on a combination of ACEI and COX-2 inhibitors, which might be more beneficial in the treatment of Alzheimer's disease than either individual agent alone.

2. Materials and Methods

2.1. *Animals*

Adult male mice weighing 25-30 g were purchased from the animal house of the National Research Center. Mice were housed in separate cages with no more than 5 animals per cage, in the laboratory animal center of the German University in Cairo, under a controlled temperature (22-23°C), on a 12-hour light/dark cycle and supplied with food and water *ad lib*. All experimental procedures were conducted according to NIH guidelines for the treatment and care of laboratory animals published by the US National Institutes of Health (NIH publication 85-23 revised 1985) and approved by the animal and human ethics committee in the German University in Cairo (GUC).

2.2. *Chemicals*

LPS (Sigma Chemical Co., USA); Perindopril (Servier Pharmaceutical Co., Egypt) and Celecoxib (Pfizer Co., Egypt) were used in this study. All were dissolved in physiological saline. TNF-α and IL-1β levels were measured using Quantikine rat TNF-α and IL-1β ELISA kits (R&D Systems).

2.3. *Experimental protocol*

Mice were divided into five groups, each containing 10 mice. The first group received a daily dose of 1% Tween 80 intraperitoneally (*i.p.*), for 7 days and served as control group. The other four groups of animals were given a single injection of LPS at a dose of 0.8 mg/kg, *i.p.* to induce an Alzheimer's model (*17,18*). In addition to LPS, the four groups received concurrent treatment for 7 days with one of the following: 1% Tween 80, *i.p.* (LPS – treated group); Celecoxib at a dose of 30 mg/kg/day, *i.p.* (LPS + Celecoxib – treated group) (*19*); Perindopril at a dose of 0.5 mg/kg/day, *i.p.* (LPS + Perindopril – treated group) (*14*) and a combination therapy of both Celecoxib and Perindopril at the same doses previously used (LPS + Celecoxib + Perindopril – treated group). At the end of the experimental protocol, behavioral tests were carried out 1 h after the last injection of the tested drugs, and then 24 h later. Animals were anesthetized with sodium phentobarbital (60 mg/kg) and killed by decapitation. Brains were rapidly removed and cut into 2 symmetrical halves by midline incision. One half was fixed in 10% formol saline for histopathological studies, and the other half was stored at –80°C for estimation of TNF-α and IL-1β.

2.4. *Behavioral experiments*

On the day of testing, mice were transported to the testing facility (Behavioral Lab, Faculty of Pharmacy, Cairo University). A 30 min period was allowed prior to testing to adapt to the environment and to minimize the effect of stress due to transfer.

2.4.1. *The open field test*

Exploratory locomotor activity was measured using the open field test (*20*). This test was carried out during the morning daylight in a quiet lab in order to avoid interference from any external stimuli. It was performed using a special a squared-shape wooden box having red sides and a white floor. Each side is 80 × 80 cm and 40 cm in height (*21*). The floor field is divided by black lines into 16 equal squares (*22*). Rats were placed individually into the central point of the open field and observed during a 3 min period for the following items:

1) *Latency*: it is the time interval (in seconds) between placing the animal at the middle of the arena until the decision of the animal to move (*23*).

2) *Ambulation frequency*: which is the number of squares crossed by the animal and was recorded per minute. The total number during the 3 min period was used to compare groups (*22,24*).

3) *Grooming frequency*: it is defined as the number during 3 min of face washing and scratching with the hind leg, licking of the fur and genitals (*21,25*).

4) *Rearing frequency*: it is the number of times the animal stood and stretched on hind limbs with or without fore limb support (*24,26*).

2.4.2. *Spontaneous alternation Y-maze test*

Immediate working memory performance was assessed by recording spontaneous alternation behavior in a Y-maze (*27*). The maze was made of black-painted wood and each arm was 25 cm long, 14 cm high, 5 cm wide and positioned at equal angles. Each mouse was placed in the center of the Y maze and allowed to explore freely during an 8-min session without reinforcers such as food, water, or electric shock. The series of arm entries were recorded visually and an arm entry was considered to be completed when the hind paws of the mouse were completely placed in the arm. The alternation behavior (actual alternations) was defined as the consecutive entry into three arms, *i.e.*, the combination of three different arms, with stepwise combinations in the sequence. The maximum number of alternations was thus the total number of arms entered minus 2, and the percentage of alternation behavior was calculated as (actual alternations/maximum alternations) × 100% (*28*).

2.5. *Histopathological studies*

2.5.1. *Histochemical study*

Cerebral specimens of control and experimental groups were placed in 10% formol saline and prepared for paraffin block sections. Five μm serial sections were cut and stained with Congo red stain (*29*). The entire procedure was performed at room temperature in a fume hood. Counterstaining was performed with haematoxylin for demonstration of nuclei. Congo red labels amyloid in brain parenchyma and blood vessels.

2.5.2. *Morphometric study*

A Leica Quin 500 LTD image analysis computer assisted system (Histology Department, Kasr Al Aini) was used. The % area of amyloid angiopathy was determined per section using an interactive measurements menu. The area of amyloid plaques was assessed. The apoptotic index was recorded as the percentage of dark nuclei in neurons.

2.6. *Biochemical parameters: TNF-α and IL-1β*

TNF-α and IL-1β, as inflammatory cytokines, were measured in the brain tissue after homogenization in ice-cold saline using a Potter-Elvejham glass homogenizer. The homogenate was centrifuged at 1,200 × g for 20 min at 4°C and the supernatant was examined for TNF-α and IL-1β using Quantikine rat TNF-α and IL-1β ELISA kits (R&D Systems) according to the manufacturer's recommendations.

2.7. *Statistical analysis*

All data are expressed as means ± S.E.M. Statistical analysis for comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by "*t*" test used as a post hoc test. The level of significance was set at $p < 0.05$. Graph Pad Software InStat (version 2) was used to carry out these statistical tests.

3. Results

3.1. *Effect of COX-2 inhibitor "Celecoxib" and ACEI "Perindopril" on LPS-induced cognitive impairment in mice*

3.1.1. *Open field test*

In the current investigation, administration of LPS (0.8 mg/kg) resulted in a significant increase in the latency period and decrease in the grooming frequency as compared to the normal control values in the open field test. Treatment with Celecoxib (30 mg/kg/day) or combined therapy of both Celecoxib and Perindopril resulted in significant protection against LPS-induced changes in both parameters ($p < 0.05$ *vs.* LPS group) (Figures 1A and 1B). Perindopril significantly decreased the latency time but had no effect on grooming frequency. In the same test LPS administration significantly suppressed the ambulation and the rearing frequencies as compared to the normal control values. Treatment with Celecoxib, Perindopril (0.5 mg/kg/day) or both drugs resulted in a significant rise in both parameters as compared to that of the LPS group (Figures 1C and 1D). All parameters were significantly different from control values ($p < 0.05$) when each drug was used alone, but were not statistically significant from control ($p > 0.05$) when a combination therapy of both Celecoxib and Perindopril was used. This indicated complete memory recovery.

3.1.2. *Y-maze test*

LPS significantly decreased the % of alternation behavior, on the other hand Celecoxib, Perindopril or the combination of both drugs significantly reversed the LPS-induced reduction of alternation behavior (Table 1; Celecoxib group, Perindopril group: $p < 0.05$ *vs.* control; Celecoxib + Perindopril group: $p > 0.05$ *vs.* control group).

3.2. *Effect of Celecoxib and Perindopril on brain histopathology in LPS-induced Alzheimer's model*

3.2.1. *Histochemical results*

Concerning the histopathological findings, sections of normal mice showed cerebral cortex exhibiting pyramidal neurons with pale nuclei surrounded by

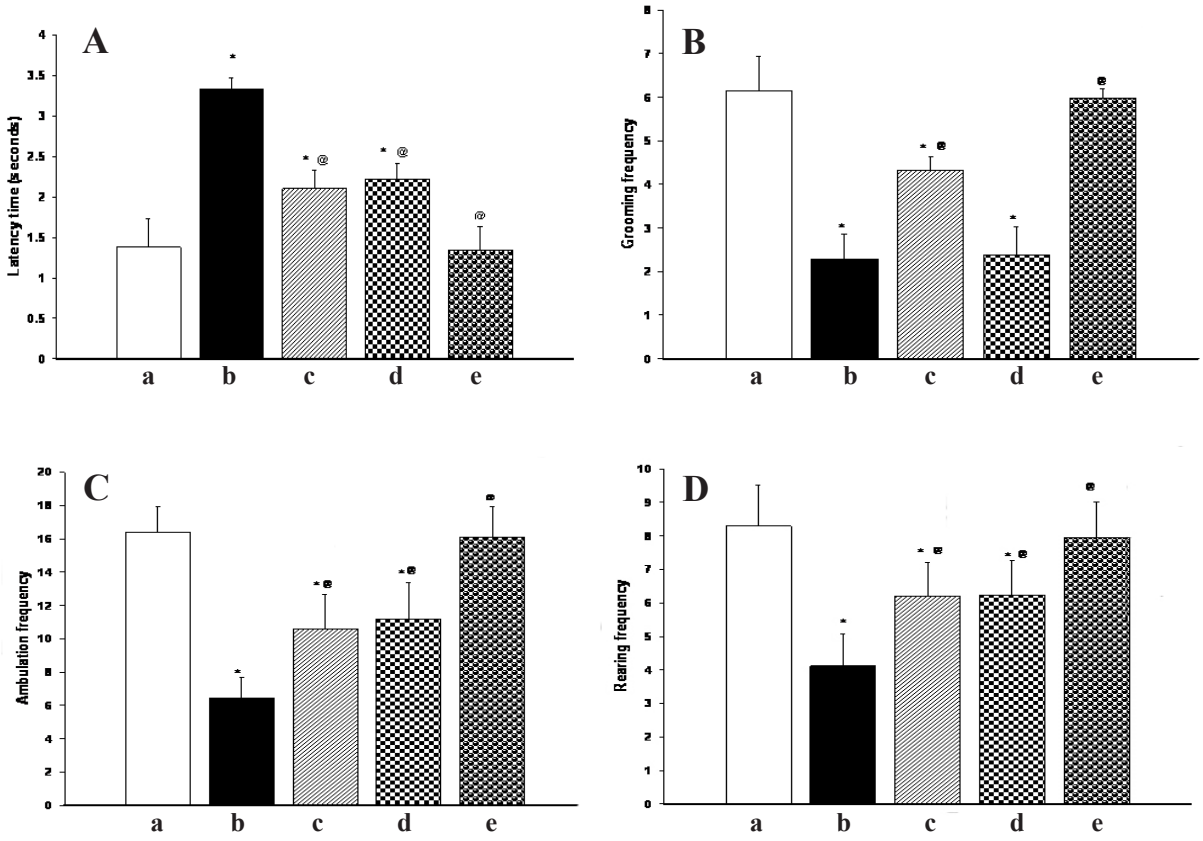

Figure 1. Effect of multiple treatment modalities on open field test in LPS-induced AD mice. Effect of lipopolysaccharide (LPS; 0.8 mg/kg, *i.p.*) with or without 7 days treatments with Celecoxib (30 mg/kg, *i.p.*), Perindopril (0.5 mg/kg, *i.p.*), Celecoxib (30 mg/kg, *i.p.*) + Perindopril (0.5 mg/kg, *i.p.*) was determined. LPS was administered as a single *i.p.* injection on the 1st day to all groups except the normal control one. The test agents were administered daily for 7 days. (**A**) Latency period of mice. (**B**) Grooming frequency of mice. (**C**) Ambulation frequency of mice. (**D**) Rearing frequency of mice. Each bar with vertical line represents the mean of 10 animals ± SE. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from LPS-treated group at $p < 0.05$. **a**, Normal; **b**, LPS; **c**, LPS + Celecoxib; **d**, LPS + Perindopril; **e**, LPS + Celecoxib + Perindopril.

Table 1. Effect of multiple treatment modalities on spontaneous alternation behavior in the Y-maze test in LPS-induced AD mice

Groups	Alternation behavior %
Normal control	75.67 ± 6.45
Control LPS	42.37 ± 3.21[a]
LPS + Celecoxib	58.34 ± 4.23[a,b]
LPS + Perindopril	59.76 ± 3.09[a,b]
LPS + Celecoxib + Perindopril	73.36 ± 4.89[b]

LPS was administered as single *i.p.* injection (on the 1st day) to all groups except the normal one. The test drugs were administered for 7 days. The control and LPS groups received 1% Tween 80 daily for 7 days. Data are expressed as mean ± SE. [a] Significantly different from control group at $p < 0.05$. [b] Significantly different from LPS-treated group at $p < 0.05$.

nerve fibers, glial cells and blood vessels (Figure 2A). In mice receiving LPS, multiple plaques formed of lamellated fibrils were observed. Such plaques were surrounded by multiple apoptotic nuclei (Figures 2B1 and 2B2). Some areas revealed blood vessels with deposits of eosinophilic material (amyloid) in their wall and extravasated red blood corpuscles around as compared to the normal control group (Figure 2B3).

Administration of Celecoxib showed few small lamellated plaques and dense plaques and less prominent apoptotic nuclei (Figure 2C1). On the other hand, Perindopril revealed less multiple lamellated plaques, some dense plaques and still multiple apoptotic nuclei (Figure 2D1). Both Celecoxib and Perindopril sections recruited vessels with minimal deposits in their walls (Figures 2C2 and 2D2). On combined therapy occasional lamellated and occasional dense plaques as well as occasional apoptotic nuclei were detected. Moreover, the blood vessels were comparable to the control group (Figure 2E).

3.2.2. *Morphometric results*

The % area of vascular angiopathy and the area of lamellated amyloid plaques were significantly reduced by Celecoxib as well as by Perindopril compared to the LPS group ($p < 0.05$), while significant reduction was recorded on combined therapy *versus* each drug alone. The apoptotic index was significantly reduced after Celecoxib treatment ($p < 0.05$ *vs.* LPS group), but Perindopril showed no significant effect ($p > 0.05$ *vs.* LPS group). Combined therapy of both drugs revealed a further significant decrease in the apoptotic index (Table 2).

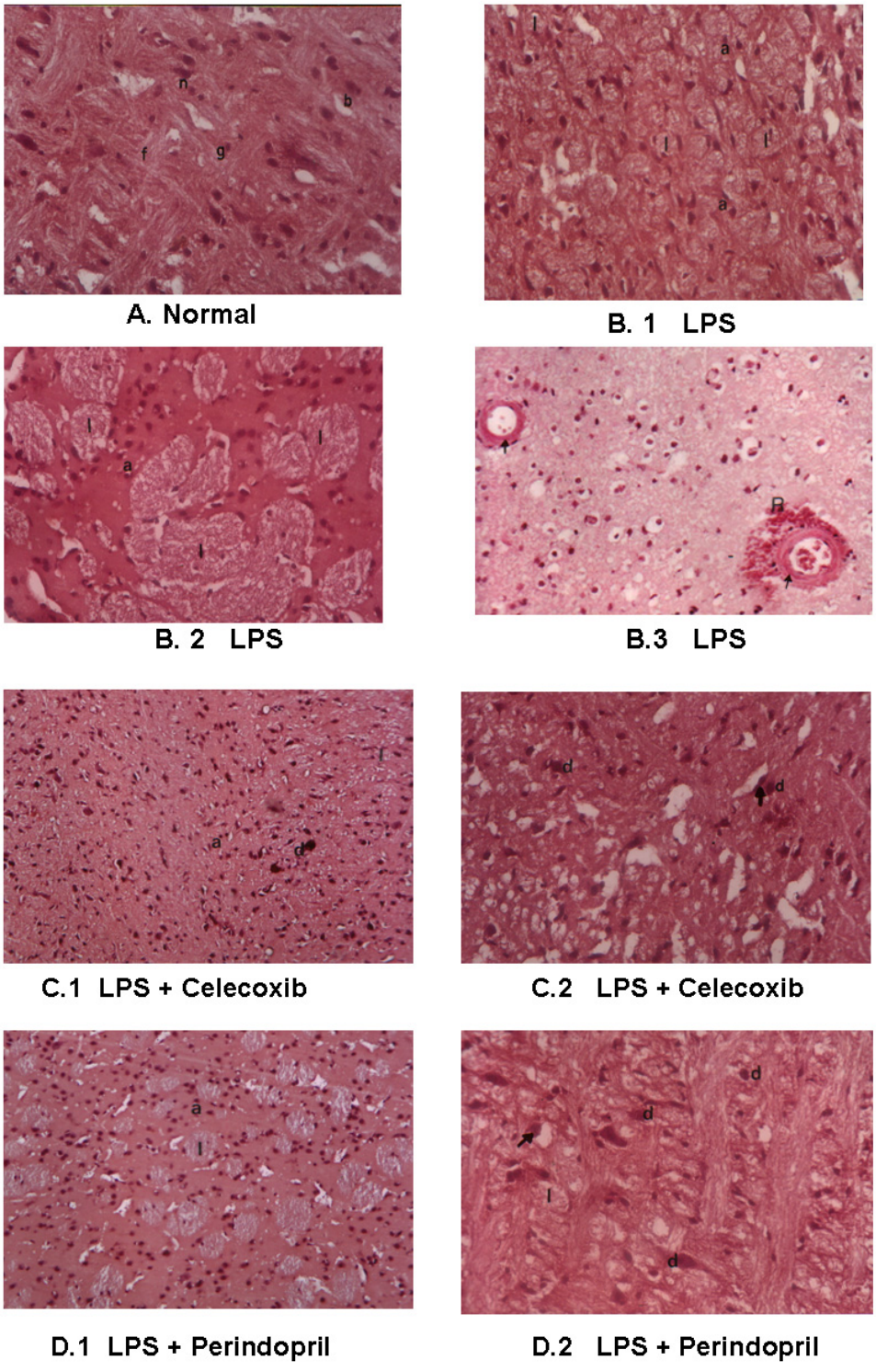

Figure 2. Effect of multiple treatment modalities on brain histopathology in LPS-induced AD mice. Photomicrograph of sections in the cerebral cortex of mice. Effect of lipopolysaccharide (LPS; 0.8 mg/kg, *i.p.*) alone (**B**) or with 7 days treatments by Celecoxib (30 mg/kg, *i.p.*) (**C**), Perindopril (0.5 mg/kg, *i.p.*) (**D**), and Celecoxib + Perindopril (**E**) on brain histopathology was determined. (**A**) Section of a control mouse showing pyramidal neurons (n), glial cells (g), nerve fibers (f), and blood vessels (b). Congo red, ×400. (**B1**) LPS-treated group showed multiple lamellated bodies (L) surrounded by apoptotic nuclei (a). Congo red, ×200. (**B2**) Higher magnification of lamellated bodies (L) and apoptotic nuclei around (a), Congo red, ×400. (**B3**) Two blood vessels with deposited eosinophilic material in their walls (arrows). Note extravasated red blood cells (R). Congo red, ×200. (**C1**) Celecoxib treatment showed few small lamellated bodies (L), some dense plaques (d) and apoptotic nuclei (a). Congo red, × 200. (**C2**) Deposited material in the wall of a capillary (arrow) and dense plaques (d). Congo red, ×400. (**D1**) Perindopril treatment showed less multiple lamellated plaques (L) and multiple apoptotic nuclei (a). Congo red, ×200. (**D2**) Same group showed lamellated plaques (L), some dense plaques (d) and deposited material in a capillary (arrow). Congo red, ×400. (**E**) Combined therapy with both Celecoxib and Perindopril showed occasional lamellated bodies (L), occasional apoptotic nuclei (a) as well occasional dense plaques (d). Congo red, ×400.

Table 2. Effect of multiple treatment modalities on % area of vascular angiopathy, area of lamellated plaques and apoptotic index in brain sections of LPS-induced AD mice

Groups	% Area of vascular angiopathy	Area of lamellated plaques	Apoptotic index
Control group	-	-	-
LPS group	4.65 ± 0.40[a]	5857.61 ± 361.5[a]	8.63 ± 0.42
Celecoxib group	1.47 ± 0.39[b]	1829.34 ± 116.49[b]	3.14 ± 0.19[b]
Perindopril group	1.68 ± 0.43[b]	1992.65 ± 52.69[b]	6.51 ± 0.60
Combined group	–	157.89 ± 32.16[b]	1.22 ± 0.11[b]

LPS was administered as a single *i.p.* injection (on the 1st day) to all groups except the control one. The test drugs were administered for 7 days. The control and LPS groups received 1% Tween 80 daily for 7 days. Data are expressed as mean of 10 animals ± SE. [a] Significantly different from control group at $p < 0.05$. [b] Significantly different from LPS-treated group at $p < 0.05$.

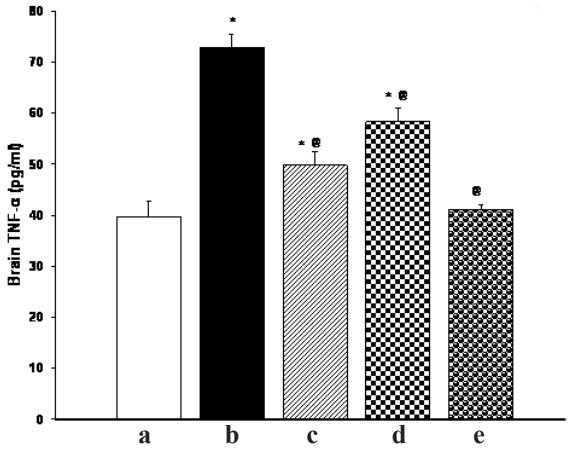

Figure 3. Effect of multiple treatment modalities on TNF-α concentration in brain homogenate samples LPS-induced AD mice. Effect of lipopolysaccharide (LPS; 0.8 mg/kg, *i.p.*) with or without 7 day treatment with Celecoxib (30 mg/kg, *i.p.*), Perindopril (0.5 mg/kg, *i.p.*), Celecoxib (30 mg/kg, *i.p.*) + Perindopril (0.5 mg/kg, *i.p.*) on the inflammatory cytokine TNF-α was determined. LPS was administered as single *i.p.* injection on the 1st day to all groups except the normal one. Each bar with vertical line represents the mean of 10 animals ± SE. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from LPS-treated group at $p < 0.05$. **a**, Normal; **b**, LPS; **c**, LPS + Celecoxib; **d**, LPS + Perindopril; **e**, LPS + Celecoxib + Perindopril.

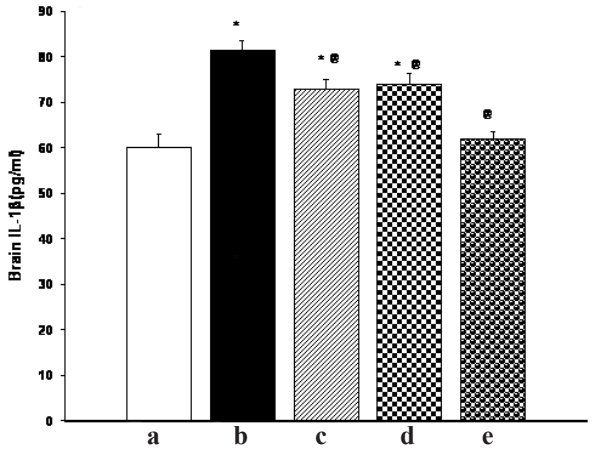

Figure 4. Effect of multiple treatment modalities on IL-1β concentration in brain homogenate samples of LPS-induced AD mice. Effect of lipopolysaccharide (LPS; 0.8 mg/kg, *i.p.*) with or without 7 day treatment with Celecoxib (30 mg/kg, *i.p.*), Perindopril (0.5 mg/kg, *i.p.*), Celecoxib (30 mg/kg, *i.p.*) + Perindopril (0.5 mg/kg, *i.p.*) on IL-1β. LPS was administered as a single *i.p.* injection on the 1st day to all groups except the normal one. Each bar with vertical line represents the mean of 10 animals ± SE. * Significantly different from normal control group at $p < 0.05$. @ Significantly different from LPS-treated group at $p < 0.05$. **a**, Normal; **b**, LPS; **c**, LPS + Celecoxib; **d**, LPS + Perindopril; **e**, LPS + Celecoxib + Perindopril.

3.3. *Effect of Celecoxib and Perindopril on TNF-α and IL-1β in AD brains*

TNF-α and IL-1β were quantified in brain supernatant extracted from the different studied groups. As shown in Figures 3 and 4, levels of both cytokines were significantly increased in the LPS treated group as compared to controls ($p < 0.05$), indicating that LPS induced a neuroinflammatory process which might contribute to AD. These cytokines were significantly attenuated after administration of either Celecoxib or Perindopril ($p < 0.05$ *vs.* LPS group) but there is still a statistical significant difference between each of these groups and the control ($p > 0.05$). Concomitant administration of the COX-2 inhibitor and the ACEI resulted in marked reduction of TNF-α and IL-1β, reaching the corresponding control values ($p > 0.05$ *vs.* control).

4. Discussion

Alzheimer's disease is the most common cause of progressive decline of cognitive function in aged humans (*30*). The pathophysiology of AD is not well understood; therefore, current therapeutic approaches for AD are merely symptomatic and offer only partial benefit without any disease modifying activity (*31*). Various complementary factors including the inflammatory cytokines (TNF and IL-1β), COX-2 as well as the renin angiotensin system seem to be involved in triggering the process of amyloidogenesis contributing to neuronal degeneration and death in AD. Our results revealed that the proposed combination therapy of both COX-2 inhibitor and ACEI proved to be more beneficial in the prevention of AD than the individual agents alone.

In the present study, we examined the effects of Celecoxib and Perindopril on LPS-induced behavioral changes in mice. The Y-maze test was employed to examine spatial learning performance based on working memory (*32*). The memory component in this task is that the mouse must remember which arm was most recently visited in order to alternate the arm choice. We observed that LPS significantly decreased spontaneous alternation, an effect that was in turn partially inhibited

by either Celecoxib or Perindopril and completely inhibited by their combination. Similar results were confirmed by Arai *et al.* (*17*), who proved that spatial memory was significantly impaired in rats injected by LPS. This suggested the induction of inducible nitric oxide synthase in the brain (*33*). The open field test has been widely used to measure behavioral responses such as anxiety-induced locomotor activity, hyperactivity, and exploratory behaviors (*34*). In our study LPS increased latency time and decreased grooming, rearing and ambulation frequencies. These locomotor changes were completely canceled by a concomitant administration of both Celecoxib and Perindopril. Similarly, Swiergiel and Dunn (*35*) reported that LPS decreased the number of line crossings in the center of the field, reflecting anxiety-like behavior. This effect was also accompanied by a similar decrease in line crossings in the periphery, as well as in rears and climbs indicating reduction in overall locomotor activity. They suggested that the ensuing cytokines could activate the hypothalamic-pituitary-adrenal axis and central noradrenergic systems and influence affective state, including anxiety-related behavior. In the present work, behavioral disturbances were accompanied by increased brain levels of both TNF-α and Il-1β in the LPS treated group. This is in agreement with previous workers who proved that peripheral administration of LPS increases the brain level of IL-1β, IL-6, and TNF-α (*6,7*) through activation of the immune system and increasing the concentrations of norepinephrine, dopamine, serotonin, and their metabolites in the hypothalamic paraventricular nucleus and in the dorsal hippocampus (*10,11*). Teeling *et al.* (*36*) found that the decrease in the locomotor behavior by LPS coincided with increased expression of cytokines in the periphery and selected regions of the brain.

Considering the concept that the AD affected brain may be in a chronic state of neuroinflammation, attempts to develop anti-neuroinflammatory compounds that attenuate neurodegeneration and improve cognitive impairment are feasible. Our study revealed that Celecoxib, a brain penetrating COX-2 inhibitor, protects against LPS-induced behavioral deficits. This has been associated with significant attenuation of TNF-α and IL-1β in the brain tissue. Our results were in agreement with the work of Cakata *et al.* (*37*), who found significant protection when using a COX-2 inhibitor in mice against memory deficit and locomotor disturbances induced by systemic administration of LPS and amyloid β.

The renin angiotensin system, already of recognized importance in the pathogenesis of hypertension, has become a source of interest in the pathogenesis of AD (*12,38*). In our study, a significant cognitive enhancing effect for the brain was observed with ACEI and Perindopril in LPS-treated mice, but it was still significantly lower than the control. A similar reduction in cognitive decline by ACEI has been reported by Hanon *et al.*, but in stroke related dementia (*39*). Jenkins and Chai (*40*) showed that groups of rats, treated with different concentrations of the ACE inhibitor, learned the location of the submerged platform in the water maze task significantly faster than control rats over 5 training days, reflecting some memory enhancing effects. This beneficial effect of ACEI could be explained by their effect on cholinergic activity *via* reduction of angiotensin II (AngII)-mediated depression of acetylcholine release (*12*). This is consistent with the documented role of the cholinergic system in learning and memory (*41*). Another possible explanation is that AngII suppresses long term potentiation, therefore, administration of ACEI could reverse cognitive deficits. On the contrary, another work demonstrated that intracerebroventricular injection of AngII enhances memory and learning suggesting a direct effect on brain angiotensin receptors including AT1 and AT4 (*42*). The current results demonstrated that Perindopril partially attenuated the LPS-induced increase in brain levels of TNF-α and IL-1β along with the improved behavioral parameters. Shimizu *et al.* (*43*) reported an involvement of peripheral AngII in the development of both fever and peripheral interleukin (IL-1β) production induced in rats by a systemic injection of lipopolysaccharide (LPS). A Miyoshi *et al.* work documented that endogenous AngII enhances LPS-induced microglial cell culture activity through stimulation of the microglial AT (*1*), which itself evokes activation of the transcription factor NF-κB (*44*). These findings could explain the inhibitory effect of Perindopril on the inflammatory response in our study.

It is interesting to note that the group of LPS-treated mice receiving concomitant administration of both Celecoxib and Perindopril displayed a complete improvement of cognitive performance as well as marked attenuation of the neuroinflammatory response. Taken together, all the previously mentioned data are indicative that each drug alone exerts a partial beneficial effect in our AD mice model and that their combination might have a synergistic effect and exert a more protective effect than each one alone. In the present work, some cerebral areas revealed blood vessels with deposited amyloid in their wall and an increased percentage area of vascular angiopathy after LPS injection. Furthermore, multiple lamellated amyloid plaques surrounded by apoptotic nuclei were observed. This is consistent with a study by Lee *et al.* who reported that systemic injection of LPS resulted in accumulation of Aβ1-42 (the major component of senile plaques of the AD brain) in both the cortex and hippocampus of mice brains accompanied with increased expression of amyloid precursor protein (*45*). A reciprocal relationship between amyloidogenesis and neuroinflammation exists (*4*), whereas certain inflammatory mediators induce and are induced by

Aβ. Therefore, in our study, we would expect that Aβ induced by LPS might stimulate glial and microglial production of IL-1 and TNF-α leading to an ongoing inflammatory cascade and contributing to synaptic dysfunction and loss, and later, neuronal death. On the other hand, increased tissue levels of IL-1 and TNF-α observed in this study could be responsible for Aβ synthesis resulting in deposition of amyloid plaques in cerebral areas and development of vascular angiopathy. In our study, the use of Celecoxib in the AD model, shows significant efficacy in decreasing the % area of vascular angiopathy with less deposition of lamellated plaques and decreased apoptotic index. Nevertheless, all these values were significantly lower that those of the control group. It has been reported that increased production of amyloid β results in vascular oxidative stress and loss of vasodilation function. The culprit molecule, superoxide, triggers the synthesis of other reactive oxygen species and the sequestration of nitric oxide (NO), which impairs resting cerebrovascular tone and NO-dependent dilation (*46*).

We observed from the present study that administration of the ACEI Perindopril partially protects the AD brain against cerebrovascular angiopathy and deposition of amyloid plaques. The apoptotic index tends to be lower than the corresponding value in the LPS group but did not reach a statistically significant level. Data from other Alzheimer models suggest that captopril and similar ACEIs do not cause Aβ accumulation *in vivo* (*15*). A more recent study by Miners *et al.* reported that ACE-1 activity is increased in AD, in direct relationship to parenchymal Aβ load and this increased ACE-1, probably of neuronal origin, accumulates perivascularly in severe cerebral amyloid angiopathy and colocalizes with vascular extracellular matrix. This effect could be attributed to increased upregulation of neprilysin (an Aβ degrading enzyme) (*48*) which also operates within the renin-angiotensin system (*16*). This possibility is further emphasized by the findings of a postmortem study that reported a significant reduction of neprilysin activity in brain homogenates of patients with AD compared to controls, and that loss of cerebrovascular-associated neprilysin in AD is inversely related to the severity of cerebral amyloid angiopathy (*49*).

A synergistic effect of both drugs, targeting wide varieties of pathophysiological mechanisms in the AD model has been observed. These data taken together with the previously discussed ones regarding complete cognitive recovery and inhibition of the neuroinflammatory cytokines support the potential use of such a combination as treatment for the pathophysiology of AD. However, there are enough clues to justify the use of such a combination as a promising strategy in treatment of AD. Many questions still remain regarding therapy.

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