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Research Article

A COMPARATIVE STUDY OF NEUROPROTECTIVE EFFECT OF TELMISARTAN AND DONEPEZIL AGAINST LIPOPOLYSACCHARIDE INDUCED NEUROINFLAMMATION IN MICE

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ABSTRACT

Objective: The aim of the present study was a comparative study of neuroprotective effect of telmisartan and donepezil against lipopolysaccharide (LPS)-induced neuroinflammation in mice.

Methods: In this study, we investigated the comparative effect of telmisartan (5 mg/kg, p.o.) and donepezil (5 mg/kg, p.o.) in systemic inflammation induced by LPS, ibuprofen (40 mg/kg, p.o.) was used as standard. Mice were treated with a single i.c. injection of LPS (5 µg/5 µl/kg), after 7 days the animal behavior was evaluated by testing specific cognitive functions, on Morris water maze and Pole climbing test. Biochemical estimation for glutathione (GSH), malondialdehyde (MDA) and tumor necrosis factor alpha (TNF-α) was done by enzyme-linked immunosorbent assay plate reader.

Results: The neuroprotective effect of telmisartan (5 mg/kg) and donepezil (5 mg/kg) in LPS induced neuroinflammation in mice was compared. Oral administration of telmisartan (5 mg/kg) for 7 days shows a better result in Morris water maze and pole climbing test, in comparison of donepezil. It also increases the level of GSH and decreases the level of MDA and TNF-α in mice brain.

Conclusion: The present study demonstrates that telmisartan and donepezil reduces LPS-induced microglial activation, beta-amyloid generation, central nervous system cytokine production, and behavioral symptoms of sickness. In comparative study of telmisartan and donepezil, telmisartan shows significant decrease in escape latency time and transfer latency time in comparison of donepezil. Therefore, telmisartan is more effective as the comparative of donepezil.

Keywords: Neuroprotective, Telmisartan, Donepezil, Lipopolysaccharide-induced neuroinflammation.

INTRODUCTION

Neuroinflammation plays an important role in the etiology of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis. In AD brains, amyloid plaques and neurofibrillary tangles, which serve as pathological markers of AD, activate microglial cells and astrocytes. In addition, tumor necrosis factor alpha (TNF-α), and interleukin (IL)-1β released by microglia can activate astrocytes, and the release of these same factors from astrocytes can lead to further activation of microglia. These pathological conditions promote the death of cholinergic neuronal cells, which results in cognitive impairment in AD patients [1]. Although acute neuroinflammation plays a protective role, chronic neuroinflammation is frequently considered detrimental and damaging to nervous tissue [2].

AD, a complex, multifactoral, progressive, neurodegenerative disease primarily affecting the elderly population is estimated to account for 50-60% of dementiacases in persons over 65 years of age. According to the World Health Organization (WHO, 2006), around 35 million people in industrialized countries will suffer from AD by 2010 [3]. The endotoxin lipopolysaccharide (LPS) is found in the outer cell wall of Gram-negative bacteria and when injected systemically can generate many features of the acute phase response and has therefore been used extensively as a model for peripherally induced inflammation. Systemic LPS causes an increase in production of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, in the periphery by immune cells such as monocytes and tissue macrophage [4]. Microglial cells represent 10% of the cells in the adult central nervous system and are morphologically characterized by small somas and ramified processes. Microglial cells also respond to foreign material such as aggregated amyloid-β [5].The brain rennin-angiotensin system (RAS) has emerged as a novel therapeutic target. Increased RAS activation, leading to excessive angiotensin 1 (AT_1) receptor stimulation, is a major factor in the development and progression of brain inflammation as a consequence of central or systemic infection, heart failure, and aging. In turn, administration of AT_1 receptor blockers (ARBs) decreases brain inflammation and is neuroprotective [6]. Telmisartan, an ARBs, is used in the management of hypertension to control blood pressure. In addition, telmisartan has a partial agonistic effect on peroxisome proliferator activated receptor γ. Recently, the effects of telmisartan on spatial memory or the inflammatory response were monitored in a mouse model of AD [7]. Donepezil ((*R,S*)-1-benzyl-4[(5,6 dimethoxy-1-indanon)-2-yl]-methyl piperidine hydrochloride) is a potent acetyl cholinesterase inhibitor. Acetyl cholinesterase inhibitors inhibit the hydrolysis of acetylcholine (ACh) and elevate its concentration in the synaptic cleft, provoking an increase of the efficacy of cholinergic neurotransmission [8]. In this study we investigated the comparative Neuroprotective effect of telmisartan and donepenzil against LPS-induced neuroinflammation in mice. Non-steroidal antiinflammatory drugs (NSAIDs) use reduces risk of dementia. One of the most popular the over the counter drug Ibuprofen is used in therapy [9].

MATERIALS AND METHODS

Drugs, chemicals, and equipments

Telmisartan from teligard tablets (Okasa), ibrufen from ibuprofen tablets (Abbot India Limited), donepezil (Sigma Life Science), LPS (Sigma Life Science), ketamine hydrochloride injection (Neon Laboratories Limited), TNF-α Kit (Gen Probe), thiobarbituric acid (TBA) (Loba Chemie), trichloroacetic acid (TCA) (Loba Chemie) and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) (National Chemicals) were used in present study. Morris water maze (SMART-CS, Panlab), Pole climbing apparatus (MEDICRAFT), Hamilton syringe, homogenizer

(IKA[R]Ti8 basic), digital timer bench top centrifugal machine (Lasin), ultraviolet (UV) spectrophotometer (Lab India) and enzyme-linked immunosorbent assay (ELISA) plate reader (BIO-RAD Imark) were also used.

Animals

The experiments were carried out with male mice weighing 22-25 g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1088/07/CPCSEA). They were kept in polypropylene cages (22.5 cm × 37.5 cm) and were maintained under standard housing conditions (room temperature, 24-27°C and humidity, 60-65%) with a 12 hrs light and dark cycle. They were allowed free access to standard pellet diet and water, *ad libitium*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures (hygia/M. Pharm/30/2012-13).

Experimental design and administration of LPS in mice

Animals were divided into five groups with six mice each.
Group I: Control group treated with vehicle for 7 days
Group II: LPS 5 µg/5 µl/kg b.w., i.c.
Group III: LPS 5 µg/5 µl/kg b.w., i.c. + telmisartan 5 mg/kg b.w., p.o. for 7 days
Group IV: LPS 5 µg/5 µl/kg b.w., i.c. + donepenzil 5 mg/kg b.w., p.o. for 7 days
Group V: LPS 5 µg/5 µl/kg b.w., i.c. + ibuprofen 40 mg/kg, p.o. for 7 days (positive control).

Adult mice were anesthetized with ketamine and fixed in a homemade frame. LPS dissolved in artificial cerebrospinal fluid (aCSF), was administered i.c. in 5 µL into the right ventricle using a micro pump equipped with a 25 µL Hamilton syringe through a needle. The electrolyte composition of aCSF was 140 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 1.2 mM Na_2HPO_4, adjusted to pH 7.4.

Behavioral tests

Morris water maze test

The male mice were used to assess the neuroprotective effect of telmisartan and donepezil in LPS induced neuroinflammation in mice. The mice were then divided into five groups of 6 mice in each group, and behavior study was done in the Morris water maze. The water maze test was performed as described by Morris *et al.* A circular pool (height: 35 cm, diameter: 100 cm) was filled with water, dyed black by dissolving food colorings and maintained at 22~25°C. An escape platform (height: 14.5 cm, diameter: 4.5 cm) was then submerged 0.5~1 cm below the surface of the water in the north-eastern quadrant of the pool. On training trials, the mice were placed in the pool of water and allowed to remain on the platform for 10 seconds and were then returned to their cage during the second trial interval. The mice that did not find the platform within 120 seconds were placed on the platform for 10 seconds at the end of trial. 24 hrs after 6 trials (two times per day for 3 days), mice were given LPS. 4 hrs after the treatment of LPS (designated as day 1), they were allowed to swim until they sought the escape platform. Escape latency, escape distance, swimming speed and swimming pattern of each mouse was monitored for 5 days (1 time/day) [10].

Pole climbing test

The male mice were used to assess the neuroprotective effect of telmisartan and donepezil in LPS induced neuroinflammation in mice. The mice were then divided into five groups of 6 mice in each group and behavior study was done in the pole climbing apparatus. The passive avoidance test is a widely accepted simple and rapid means of memory testing. Passive avoidance response was determined using a "step-through" apparatus which consisted of an illuminated and dark compartment (each 20.3 cm × 15.9 cm × 21.3 cm) adjoining each other through a guillotine door. Floors were constructed of 3.175 mm stainless steel rods set 8 mm apart. The test was conducted for 2 consecutive days at the same time each day. On the first day (learning trial) each mouse was placed in the illuminated compartment facing away from the dark compartment. Once the mouse enters completely into the dark compartment, it receives an electric shock (1 mA, 3 seconds) through the stainless steel grid floor. The amount of time it took for the mouse to enter into the dark compartment was recorded automatically and described as step-through latency. On the second day (testing trial), the same test procedure was followed. When the mouse did not enter the dark compartment within 30s; the test was terminated and a latency of 30 seconds was recorded [11].

Biochemical estimation of markers of oxidative stress

Preparation of homogenate

At the end of the study period, animals were sacrificed by deep ether anesthesia. Whole brains were quickly dissected out, washed with ice-cold sodium phosphate buffer, weighed, and stored over ice. The brains were further processed within ½ hr of dissection and the estimation of oxidative stress done on the same working day. Brain tissue was homogenized in a homogenizer with 10 times (w/v) sodium phosphate buffer (pH 7.4 ice-cold mixture of KH_2PO_4 and Na_2HPO_4). The homogenate was centrifuged in a digital timer bench top centrifugal machine at 3,000 rpm for 15 minutes, and the supernatant was used for the estimation of malondialdehyde (MDA), reduced glutathione (GSH) and TNF-α [12].

Estimation of reduced GSH

Reduced GSH in brain was estimated according to the method described by Ellman (1959). A 1 ml supernatant was precipitated with 1% TCA for 5-10 minutes. The samples were centrifuged at 1200 g for 15 minutes at 4°C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of DTNB were added. The yellow color developed was read immediately at 412 nm using UV spectrophotometer. The GSH level was expressed as µg/mg protein [13].

Estimation of MDA

The MDA level was determined by a method based on the reaction with TBA at 90-100°C (Esterbauer and Cheeseman, 1990). In the TBA test reaction, MDA or MDA like substances and TBA react together for the production of a pink pigment having an absorption maximum of 532 nm. The reaction was performed with pH 2-3 at 90°C for 15 minutes. The sample was mixed with 2 volumes of cold 10% (w/v) TCA to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 minutes. After cooling, the absorbance was read at 532 nm (UV spectrophotometer, Lab India). The MDA level was expressed as nmol/mg protein [14].

Estimation of TNF-α

TNF-α concentrations were measured in supernatant of astrocytes using ELISA specific for mice TNF-α, respectively, according to the manufacturer's instructions. After samples and standards were added to wells, plates were incubated for 1 hr at 37°C. Wells were washed 7 times with wash solution, at which point antibody was added to each well and incubated for 30 minutes at 4°C. After two additional wash procedures, substrate solution was added to each well, and plates were further incubated for 30 minutes at room temperature in the dark, at which point stop solution was added to all wells. Finally, the resulting color was assayed at 450 nm using a microplate absorbance reader [14].

Statistical analysis

The results are expressed as mean±standard error of the mean, n=6. The results obtained from the present study were analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests

and Bonferroni's multiple comparison tests. Data was computed for statistical analysis by using Graph Pad Prism 5.0 software.

RESULTS

Effect of donepezil and telmisartan on LPS induced cognitive deficits in Morris water maze test

In control groups, a significant decrease in escape latency time during 4th and 5th sessions was observed in comparison to session 1; however, administration of LPS caused memory impairment as there was no significant change in latency time throughout all the water maze sessions (Fig. 1). Treatment with standard NSAIDs drug Ibuprofen prevented LPS induced neuroinflammation as indicated by significant reduction in latency time during 3rd, 4th and 5th sessions in comparison to session 1. Treatment with telmisartan and donepezil at 5 mg/kg dose for 7 days showed neuroprotective effect in LPS induced neuroinflammation in mice. Telmisartan and donepezil significantly decreased escape latency time from 4th session onward. Telmisartan shows more effective result in the comparison of donepezil (Fig. 1).

Effect of donepezil and telmisartan on LPS induced cognitive deficits in pole climbing test

There was a significant decrease in transfer latency time on days 4 and 5 as compared to day 1 in control group (Fig. 2). However, administration of LPS caused memory impairment as indicated by no significant change in pole climbing latency throughout all the days. Treatment with telmisartan and donepezil at 5 mg/kg dose for 7 days showed significant decrease in latency to climb pole on day 4 and 5 in comparison to day 1. The standard NSAIDs drug Ibuprofen showed significant reduction in pole climbing latency indicating amelioration of LPS induced neuroinflammation in mice. Telmisartan and donepezil significantly decreased transfer latency time from 4th session onward. Telmisartan shows more effective result in the comparison of donepezil (Fig. 2).

Effect of donepezil and telmisartan on GSH level

GSH (µg/mg protein) was estimated in mice brain after the completion of behavioral studies. As shown in Fig. 3, a significant fall in the levels of GSH was observed in LPS group as compared to the control. The standard NSAIDs drug Ibuprofen showed significant increase in GSH level indicating amelioration of LPS induced neuroinflammation in mice. There was significant rise in the level of GSH in the group treated with telmisartan (5 mg/kg) and donepezil (5 mg/kg) as compared to LPS control. In the comparison of telmisartan and donepezil, telmisartan shows the more effective result.

Effect of donepezil and telmisartan on MDA level

The MDA level (nmol/mg protein) was estimated in mice brain after the completion of behavioral studies. The MDA level rose significantly in brain of LPS treated mice as compared to control group. On the other hand, Ibuprofen significantly decreased the MDA level in comparison to LPS group. The standard NSAIDs drug Ibuprofen showed significant reduction in MDA level indicating amelioration of LPS induced neuroinflammation in mice. Treatment with telmisartan (5 mg/kg) and donepezil (5 mg/kg) significantly decreased MDA level as compared to LPS control. On the basis of MDA level, telmisartan is more effective as compared to donepezil (Fig. 4).

Effect of donepezil and telmisartan on level of TNF-α

The TNF-α level was estimated in mice brain after the completion of behavioral studies. LPS administration by i.c. route significantly increased the level of TNF-α as compared to control. The standard NSAIDs drug Ibuprofen showed significant reduction in TNF-α level

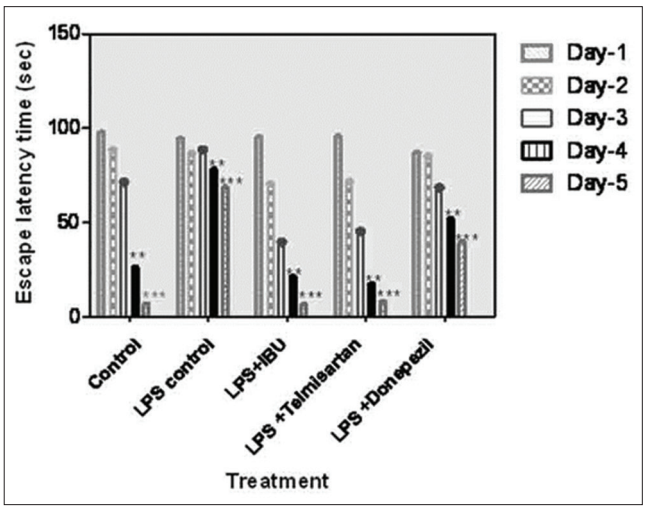

Fig. 1: Effect of telmisartan and donepezil on lipopolysaccharide induced memory impairment in mice using Morris water maze test. Data values are expressed as mean escape latency time (second)±standard error of mean. **p<0.05 and ***p<0.001 (significantly different from the control in comparison to session 1 of respective groups)

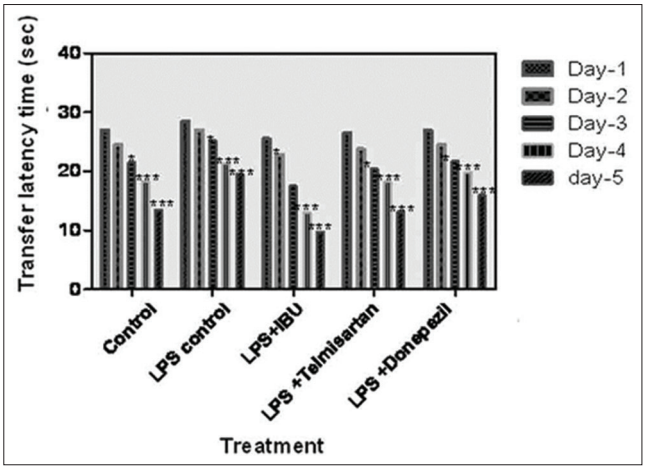

Fig. 2: Effect of telmisartan and donepezil on lipopolysaccharide induced memory impairment in mice using Pole climbing test. Data values are expressed as mean transfer latency time (second)±standard error of mean. **p<0.05 and ***p<0.001 (significantly different from the control in comparison to session 1 of respective groups)

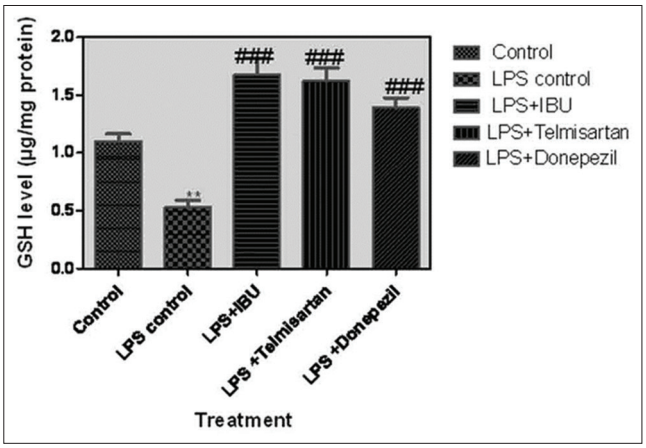

Fig. 3: Effect of telmisartan and donepezil on glutathione level in mice brain. Data values are expressed as mean glutathione level (µg/mg protein)±standard error of mean. Each group expressed *p<0.05, **p<0.01, ###p<0.001 in comparison to lipopolysaccharide control group

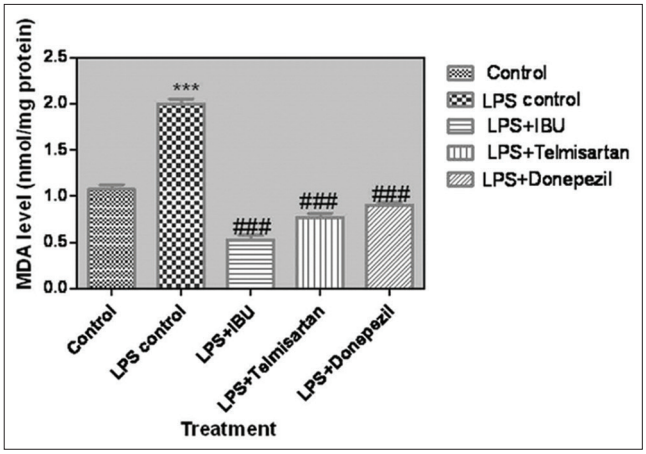

Fig. 4: Effect of telmisartan and donepezil on malondialdehyde level in mice brain. Data values are expressed as mean malondialdehyde level (nmol/mg protein)±standard error of mean. Each group expressed ###p<0.001 and ***p<0.001 in comparison to lipopolysaccharide control group

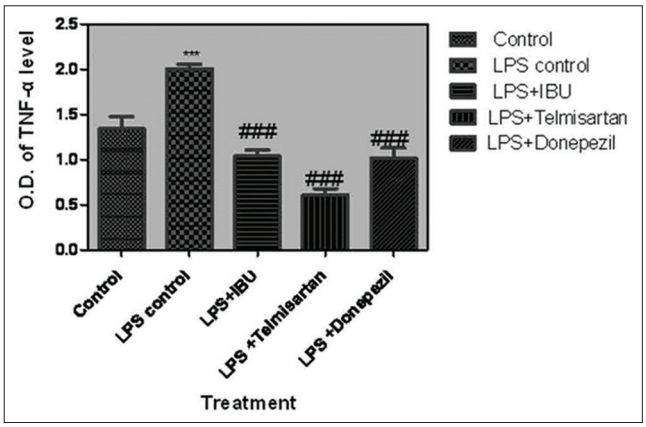

Fig. 5: Effect of telmisartan and donepezil on tumor necrosis factor alpha. Data values are expressed as mean± standard error of mean. Each group expressed ###p<0.001, ***p<0.001, *** p<0.05 in comparison to lipopolysaccharide control group

indicating amelioration of LPS induced neuroinflammation in mice. On the other hand, both Telmisartan (5 mg/kg) and donepezil (5 mg/kg) significantly decreased TNF-α level as compared to LPS control (Fig. 5).

DISCUSSION

Neuroinflammation mediated by the activation of microglia or astrocytes plays an important role in the pathology of neurodegenerative disorders such as AD and Parkinson's disease. Activated microglia releases pro-inflammatory cytokines, including TNF-α, and various ILs, as well as prostaglandins, leukotrienes, and NO. Brain microglias are activated in certain stages of neurodegenerative diseases and in young adult subjects when the peripheral innate immune system is stimulated with LPS. Activated microglia produces inflammatory cytokines that are partially responsible for the progression of neurodegenerative diseases and for LPS-induced sickness behavior. LPS is derived from Gram-negative endotoxin bacterium that acts through a membrane bound receptor complex found on the surface of microglia. Its primary action initiates nuclear translocation of transcription factor nuclear factor κB and the upregulation of proinflammatory gene expression. Following injection into the midbrain, LPS has been shown to activate microglia, increase pro-inflammatory cytokine levels. The cholinergic hypothesis of AD concludes that the cognitive deterioration that occurs with the disease is associated in part with progressive loss of cholinergic neurons and decreasing ACh levels in the brain. AT_2 stimulates two receptor types, the AT_1 and AT_2 receptors. Excessive AT_1 receptor stimulation is associated with brain inflammation, whereas stimulation of AT_2 receptors has been proposed to exert balancing neuroprotective effects, particularly when AT_1 receptors are blocked by ARB administration. Telmisartan is a new, orally active, non-peptide antagonist of the AT_1 receptor, which has been developed for the treatment of hypertension. Telmisartan is an ARB that is highly selective for the AT_1 receptor. In addition to its vasodilatory properties telmisartan appears to exert a further antihypertensive effect by directly modulating renal excretory function.

In the present study, cholinergic system also got affected in LPS induced memory deficit because of enhancement of activity of AChE resulting in increased degradation of ACh. Telmisartan devoid of any inherent anti-AChE activity brought the level of AChE in LPS treated mice to the value of control animals. ARBs exhibit anti-oxidant action as evidenced in this study where ARBs telmisartan reduced lipid peroxidation product MDA, TNF-α and elevated antioxidant, i.e. GSH. Antioxidant action of ARBs is due to blockade of AT_2 binding to AT_1 receptors which activates NADPH oxidase.

On the basis of above data, comparative neuroprotective effect of telmisartan and donepezil on Morris water maze, Pole climbing test and biochemical estimation of GSH, MDA and TNF-α showed that telmisartan is more effective in comparison of donepezil on LPS-induced neuroinflammation and cognitive deficits in mice.

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