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Revisiting the systemic lipopolysaccharide mediated neuroinflammation: Appraising the effect of L-cysteine mediated hydrogen sulphide on it

Abdulaziz S. Al-Saeedan [a,1], Varsha Gautam [b,1], Mohd Nazam Ansari [a,*], Manjari Singh [b], Rajnish K. Yadav [b], Jitendra K. Rawat [b], Uma Devi [c], Swetlana Gautam [b], Subhadeep Roy [b], Gaurav Kaithwas [b]

[a] Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia
[b] Department of Pharmaceutical Sciences, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, UP, India
[c] Department of Pharmaceutical Sciences, FHMSIASM SHIATS-Deemed University (Formerly Allahabad Agriculture Institute), Allahabad, UP, India

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ABSTRACT

The present research was ventured to examine the effect of L-cysteine on neuro-inflammation persuaded by peripheral lipopolysaccharides (LPS, 125 μg/kg, i.p.) administration. No behavioral, biochemical, and inflammatory abnormality was perceived in the brain tissues of experimental animals after LPS administration. L-cysteine precipitated marginal symptoms of toxicity in the brain tissue. Similar pattern of wholesome effect of LPS were perceived when evaluated through the brain tissue fatty acid profile, histopathologically and NF-κBP65 protein expression. LPS was unsuccessful to alter the levels of hydrogen sulphide (H_2S), cyclooxygenase (COX) and lipoxygenase (LOX) enzyme in brain tissue. LPS afforded significant peripheral toxicity, when figured out through inflammatory markers (COX, LOX), gaseous signaling molecules nitric oxide (NO), H_2S, liver toxicity (SGOT, SGPT), and inflammatory transcription factor (NF-κBP65) and L-cysteine also provided a momentous protection against the same as well. The study inculcated two major finding, firstly LPS (i.p.) cannot impart inflammatory changes to brain and secondly, L-cysteine can afford peripheral protection against deleterious effect of LPS (i.p.)

1. Introduction

Hydrogen sulphide (H_2S) is a colorless gas, with a consideration of having no physiological importance, until recently being renowned as third most important gaseous signaling molecule respectively followed by nitric oxide (NO) and carbon monoxide (CO) (Abe and Kimura, 1996; Wang, 2002; Martelli et al., 2012). The H_2S has gained importance as a bioactive molecule with several regulatory effects on biological systems including brain. In fact, comparatively high concentration of H_2S has been recorded in the brain tissues of mammals and rodents, which further strengthens the importance of H_2S in neuroscience (Goodwin et al., 1989; Savage and Gould, 1990). Indeed, inorganic H_2S donors have been extensively studied in variety of experimental neuronal disorders.

The biosynthetic pathway of H_2S involves the use of L-cysteine and homocysteine as a substrate. The intracellular pools of free cysteine are maintained by diverse mechanisms and in experimental system H_2S/H_2S donors have demonstrated various physiological protections particularly for the cardiovascular, gastrointestinal and central nervous system (Wang, 2012). The H_2S donors are also reported to reduce inflammation through variable mechanisms including inhibition of neutrophil adhesion and tissue infiltration, decrease in production of reactive oxygen species (ROS) and inflammatory cytokines (Gupta et al., 2010).

It is worth to mention that H_2S is also considered being a broad spectrum poison and having the potential to down regulate several systems of the body, among which the central nervous system is most vulnerable. The toxic effects of H_2S are due to its ability to complex with iron in mitochondrial cytochrome enzymes with subsequent curtailment of cellular respiration (Gerasimon et al., 2007).

* Corresponding author at: Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, PO Box 173, Al-Kharj 11942, Saudi Arabia.
E-mail address: m.ansari@psau.edu.sa (M.N. Ansari).
[1] Both contributed equally.

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Considering the above H_2S donors are nowadays suggested/evaluated in various pathologies and on the same line L-cysteine has been evaluated in variable preclinical setups. Nonetheless there are variable reports with few citing it is neurotoxic and some favoring it as neuroprotective (Gu et al., 2000; Janaky et al., 2000; Hashimoto et al., 2004)

A diversified range of mechanisms have been proposed for the neurotoxic as well as neuroprotective effects of L-cysteine and therefore, requires a strict reconsideration before further recommendations. While going through most of studies associated with L-cysteine, it was apparent that there was large variability in the dose selection. Therefore, the authors are in opinion that the dose of L-cysteine is the key player for the variable effects of L-cysteine observed by various researchers.

Henceforth, the further use of L-cysteine needs a careful randomization of its dose. Subsequently, the recent research was undertaken to elucidate the consequence of variable dose of L-cysteine contrary to the neuroinflammatory changes in albino rats.

2. Materials and methods

2.1. Drugs and chemicals

L-cysteine was acquired from Himedia Laboratories, Mumbai, India and lipopolysaccharide (LPS) was purchased from Santa Cruz Biotechnology, Texas75220, USA. SGPT and SGOT kits were procured from Erba Diagnostics Inc, Mumbai, India. All other chemicals were of analytical grade and obtained from Himedia Laboratories, Mumbai, India else otherwise specified in the text.

2.2. Animals

The male Wistar albino rats (100–120 g) were acquired from central animal house. The animals were kept under standard laboratory conditions of normal temperature (25 ± 1 °C) and with a light/dark cycle of 12 h with free access to commercial pellet diet and water *ad libitum*. Animals were accustomed to laboratory conditions for seven days before the experiment. The study follows the guidelines laid by committee for the purpose of control and supervision of experiment in animals, Government of India, with approval number (SDCOP & VS/AH/CPCSEA/01/0029).

2.3. Experimental design

Rats were arbitrarily distributed into five groups (n = 6) and subjected to the treatment for 28 days as elaborated afterwards. Group I as control (normal saline, 3 ml/kg, p.o.); group II: toxic control (LPS, 125 μg/kg, i.p.); group III [L-cysteine, 25 mg/kg, p.o. + LPS, 125 μg/kg, i.p.]; group IV (L-cysteine, 12.5 mg/kg, p.o. + LPS, 125 μg/kg, i.p.); group V [L-cysteine, 6.5 mg/kg, p.o. + LPS, 125 μg/kg, i.p.)]. Neurotoxicity was induced through the administration of LPS (125 μg/kg, i.p) for the four consecutive days, starting four days and last four days.

2.4. Evaluations

2.4.1. Neurobehavioral analysis

The neurobehavioral analysis in Wistar albino rats were analyzed using the elevated plus maze (EPM), rotarod test, and locomotor activity method on 17th, 21st, and 22st day of the study respectively. The detail procedure was followed as described by our laboratory (Yadav et al., 2016; Tiwari et al., 2016).

2.4.2. Biochemical assays

After 28 days of treatment, blood samples were collected in pre-heparinized tubes though retro-orbital plexus using chloroform anesthesia. The blood samples were centrifuged at 10,000 rpm to collect plasma. Animals were sacrificed through light ether anesthesia pursued by heart perfusion. Brain tissues were evacuated rapidly and sensibly escaping damage, rinsed in ice-cold saline and dried out on filter paper. Brain tissue homogenates (10% ice cold KCl) were prepared and centrifuged at 10,000 rpm for 10 min at 4 °C in cooling centrifuge and separated the supernatant. Tissue supernatant was used for the biochemical estimations of thiobarbituric reactive substances (TBAR's), superoxide dismutase (SOD), glutathione (GSH), catalase, protein carbonyl (PC) and acetylcholine-esterase (AchE) by using the procedure previously standardized at our laboratory (Reznick and Packer, 1994; Kaithwas et al., 2011; Kaithwas and Majumdar, 2012).

2.4.3. Estimation of SGOT and SGPT in blood plasma

The activity of hepatic enzymes SGOT, and SGPT was determined in plasma samples as method described by the manufacturer using colorimetric kits.

2.4.4. Estimation of tissue H_2S

The zinc precipitation method for sulphide determination was followed with slight modifications. Concisely, 500 μl of tissue supernatant was added to 400 μl of premixed zinc acetate (350 μl, 1% w/v) solution and 50 μl of sodium hydroxide (1.5 M) followed by centrifugation. The collected pellets were re-suspended with 160 μl of Milli-Q water and mixed with 40 μl of dye (20 μl of 20 mM NNDP in 7.2 M HCl and 20 μl of 30 mM $FeCl_3$ in 1.2 M HCl). The mixture was incubated at 37 °C for 10 min and read absorbance at 670 nm using UV spectrophotometrically (Carry60, Agilent Technologies, CA 95051, USA) (Ang et al., 2012).

2.4.5. Estimation of plasma H_2S

Methylene Blue method was used for the determination of plasma H_2S level with slight modification as reported by our laboratory (Tiwari et al., 2016).

2.4.6. Determination of Nitric Oxide (NO) level

Production of NO in the plasma samples was interceded by quantifying nitrite accumulation, using Griess reagent. The same quantity (500 μl) of plasma and Griess reagent was added followed with incubation at 37 °C for 5 min. Subsequently, the absorbance of resultant mixture was read on UV–Visible spectrophotometer at 540 nm (Cary 60, Agilent technologies, CA95051, USA) (Giustarini et al., 2008).

2.4.7. Fatty acid methyl ester (FAME) analysis of brain tissue

Brain tissue homogenate (0.05%) was prepared in the mixture of chloroform: methanol (2:1). The tissue homogenate was subsequently filtered with a Whatman filter paper. A 4 ml of the double distilled water was added to the filtrate to remove the non-lipid contaminates. The mixture was allowed to settle down for 30 min followed by centrifugation. The upper phase thrown out and lower phase carried the brain lipid used for preparation of methyl esters.

The lower phase (0.5 g) mix well in hexane (2 ml) and subsequently, 2 N methanolic KOH (0.2 ml) was added to the above mixture and vortexed for 15 min. The phases were allowed to settle down and the upper layer containing the FAME was collected. Furthermore, the FAME analysis was performed as reported by our laboratory (Folch et al., 1957; Kaithwas et al., 2011).

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