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Increase in seizure susceptibility in sepsis like condition explained by spiking cytokines and altered adhesion molecules level with impaired blood brain barrier integrity in experimental model of rats treated with lipopolysaccharides

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ABSTRACT

Background and objective: Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures. Sepsis is a condition which initiates a cascade of a surge of inflammatory mediators. Interplay between seizures and inflammation other than of brain origin is yet to be explored. The present study was designed to evaluate the seizure susceptibility in experimental models of lipopolysaccharide (LPS) induced sepsis.

Design and methods: Experimental sepsis was induced using lipopolysaccharides in Wistar rats. Valproic acid, dexametasone were given to two different groups of animals along with LPS. Two groups of animals were subjected to administration of vehicle and LPS respectively with no other treatment. 24 h later, animals were subjected to seizures by using either maximal electro shock or pentylenetetrazole. Seizures related parameters, oxidative stress and TNF-α, IL-6, IL-1β, ICAM-1, ICAM-2, VCAM-1, MMP-9 level in serum and brain samples were evaluated. Histopathological and blood brain barrier permeability studies were conducted.

Results: Seizures were decreased in valproic acid treated animals. Reduced oxidative stress was seen in dexamethasone plus valproic acid treated groups as compared to LPS alone treated group. TNF-α, IL-6, IL-1β, ICAM-1, VCAM-1, MMP-9 levels were found increased in LPS treated animals whereas a reverse observation was noted for ICAM-2 level in brain and serum. Histopathological findings confirmed the successful establishment of sepsis like state in animals. Blood brain barrier permeability was found increased in LPS treated groups of animals.

Conclusion: Seizure susceptibility may escalate during the sepsis like inflammatory conditions and curbing the inflammatory state might reverse the phenomenon.

1. Introduction

Sepsis represents a systemic inflammatory response syndrome induced by an infection (Robertson and Coopersmith, 2006). Sepsis results from an overwhelming systemic host inflammatory response to infection. Such inflammation is triggered by profuse release of cytokines into the systemic circulation. An understanding of the role of inflammatory processes, particularly of the cytokines, in the clinical presentations and neuropathology of seizures has been evolving. Role of pro-inflammatory and anti-inflammatory cytokines in seizures has been elucidated in past few years (Vezzani et al., 2004). Results of the recent studies in experimental models and clinical settings signal the contribution of inflammatory processes in the brain to the etiopathogenesis of epilepsy (Vezzani and Granata, 2005). It has been shown that cytokine (IL- 1β) activates neuronal IL-1R1 which leads to phosphorylation of NR2 B subunit of NMDA receptor. This results in NMDA mediated calcium influx into neurons promoting excitotoxicity (Viviani et al., 2003). Cytokines not only decrease the reuptake of glutamate by astrocytes which increases its extracellular concentration (Ye and Sontheimer 1996; Hu et al., 2000) but also induce glutamate release

Abbreviations: ANOVA, analysis of variance; BBB, blood brain barrier; SSA, sulphosalicylic acid; NBT, nitro blue tetrazolium; CNS, central nervous system; CPCSEA, Committee for the purpose of control and supervision of Experiments on animals; DDW, Double distilled water; Dexa, Dexamethosone; EDTA, Ethylene diamine tetra acetic acid; ELISA, enzyme linked immunosorbant assay; GSH, reduced glutathione; H & E, hematoxylin and eosin; IAEC, institutional animal ethics committee; ICAM, intercellular adhesion molecule; IL, interleukin; LPO, lipid peroxidation; LPS, lipopolysaccharide; MDA, malondialdehyde; MES, maximal electro shock; MMP, matrix metalloproteinase; PBS, phosphate buffer saline; PGIMER, post graduate institute of medical education and research; SEM, standard error of mean; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloracetic acid; TNF-α, tumour necrosis factor-α; VCAM, vascular cell adhesion molecule; Veh, vehicle; VA, valproic acid

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from glia by producing TNF-alpha (Bezzi et al., 2001). An elevated extracellular glutamate level leads to the genesis or spread of seizure like events (Tian et al., 2005). IL-1β has also been known to contribute to excitotoxicity by compromising GABA mediated inhibitory synaptic transmission (Wang et al., 2000). Cytokines have the potential to increase vascular permeability of BBB by damaging the tight junction and producing nitric oxide. This renders the neuronal environment hyperexcitable (Oby and Janigro, 2006). It is becoming increasingly ostensible that inflammation plays an important role in some of the damage and reorganization that is observed following a variety of brain insults such as hypoxia/ischemia, traumatic brain injury, infection etc. (Vezzani et al., 2011).

There is an immediate surge of cytokines within few hours of the administration of sepsis inducing agents in experimental models of sepsis in animals. An increased level of circulating cytokines such as TNF-α, IL-6, IL-12 and migration inhibitory factor (MIF) of macrophage is documented in central nervous system (CNS) inflammation and increased level of cytokines in CNS are directly correlated with the increase in seizure susceptibility.

Although much of the published work points at neuro-inflammation being strongly related to seizures, a direct investigation of the role of increased levels of cytokines seen due to peripheral inflammation i.e other than central nervous system, e.g. sepsis, in seizure susceptibility is not yet fully elucidated

Corticosteroids are produced in response to inflammatory cytokines as an immunomodulatory feedback mechanism (Besedovsky et al., 1986) and display wide ranging anti-inflammatory properties (e.g., inhibition of production of pro-inflammatory cytokines, free radicals, prostaglandins and inhibition of chemotaxis, and adhesion molecule expressions). There are a number of possible ways by which corticosteroids contain this inflammatory condition in sepsis. To begin with, glucocorticoids diffuse through cell membrane and interacts with the glucocorticoid receptor and forms a dimer which then enters the nucleus. The dimer interacts with glucocorticoid responsive elements (GREs), some of which downregulate the transcription of pro-inflammatory genes e.g. cytokines, adhesion molecules (Paliogianni et al., 1993). Corticosteroids also inhibit NF- kB dependent gene transcription by inducing inhibitor of NF-kB (I-kB) (Scheinman et al., 1995).

Corticosteroids including dexamethasone have proven efficacy for restoring homeostasis and vital organ functions impaired during sepsis or septic shock (Annane, 2011). Valproic acid is effective in inhibiting seizures in a variety of models of seizures. Valproic acid abolishes the hind limb tonic extension in maximal electroshock seizures and kindled seizures at nontoxic doses. Like ethosuximide, valproic acid at subtoxic doses inhibits clonic motor seizures induced by pentylenetrazole. Its efficacy in diverse animal models simulates its efficacy against absence as well as partial and generalized tonic-clonic seizures in humans (Loscher, 2002).

Therefore, the present study was set out to bridge the gap between two diverging sets of research areas where interplay between the high cytokines level induced by sepsis and the seizure susceptibility was to be mined. Sepsis was induced experimentally in animals with lipopolysaccharides. Thereafter, those animals were treated with corticosteroid Dexamethasone (DMA) and a standard anti-epileptic agent Valproic Acid (VA) to study their overall response towards identifying possible future therapeutic interventions in managing seizures precipitated as a result of systemic infections not pertaining to the brain.

2. Materials & methods

2.1. Animals in experiments

All experimental work was conducted after approval (Reference No 57/IAEC/309) from Institutional Animal Ethics Committee (IAEC) of Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for approval. Experiments were carried out on 102 Wistar rats of adult age group having weight between 150 and 200 g and either sex. Animals were procured from advanced facility for small animal research, PGIMER, Chandigarh. The animals were housed in polypropylene cages under controlled temperature and humidity environment with 12- hours light/dark cycle conditions with free access to food and water *ad libitum*. Before commencing the experiments, animals were allowed to acclimatize to laboratory environment for seven days followed by their segregation into different experimental groups.

2.2. Drugs and chemicals

LPS from *Escherichia coli* 0111:B4 was purchased Sigma Aldrich, India. Valproic acid (VA) was provided by Provizer pharma as a gift sample. Bovine fibrinogen and albumin, Tris–HCl, trichloroacetic acid (TCA), sulphosalicylic acid (SSA), nitro blue tetrazolium (NBT), ethylenediamine tetrachloroacetic acid (EDTA), sodium carbonate, hydroxylamine hydrochloride, sulphanilamide, phosphoric acid, napthylamine diamine dihydrochloric acid, sodium phosphate buffer, acetylthiocholine iodide, sodium azide, thiobarbituric acid (TBA), Tris-HCl buffer and hydrogen peroxide were bought from Central drug house pvt. ltd., New Delhi. TNF-α ELISA kits were purchased from Diaclone. IL-6, IL-1β, ELISA kits were purchased from Ray Biotech. ICAM-1, ICAM-2, VCAM-1, MMP-9 ELISA kits were purchased from Qayee- Bio.

2.3. Experimental design

Animals were divided into 8 groups each comprising of twelve animals. 4 groups were allocated to each pentylenetetrazole (PTZ) and maximal electro smalls (MES) methods of seizure induction. Six animals from each group were subjected to Blood brain barrier (BBB) permeability studies.

2.3.1. PTZ-Vehicle group

In this group, rats were given 0.25 ml of Phosphate Buffer Saline (PBS) through intraperitoneal route. PTZ seizure test was done after 24 h of PBS injection. Animals were given ether anesthesia. Animals were sacrificed under anesthesia and blood was collected by cardiac puncture for cytokines and biochemical estimation (lipid peroxidation, SOD, GSH, catalase). Brain tissue was collected for estimation of cytokines and biochemical parameters (lipid peroxidation, SOD, GSH, catalase). Liver, Kidneys and lungs were fixed in 10% aqueous formaldehyde for histopathological examination.

2.3.2. PTZ-LPS group

In this group, rats were given 10 mg/kg of LPS through intraperitoneal route. PTZ seizure test was done after 24 h. Animals were given ether anesthesia. Animals were sacrificed under anesthesia and all the assessments were carried out as in group AI.

2.3.3. PTZ-LPS + Dexa group

In this group, dexamethsone at the dose of 10 mg/kg per day was given through intraperitoneal route. After one hour of administration of dexamethasone, LPS at the dose of 10 mg/kg through intraperitoneal route was given. PTZ seizure test was done after 24 h after repeating another dose of dexamethasone half an hour prior to seizure test. Animals were given ether anesthesia.All the assessments were done as in group A I.

2.3.4. PTZ-LPS + VA group

In this group, valproic acid at the dose of 250 mg/kg was given through intraperitoneal route. After one hour of administration of valproic acid, LPS at the dose of 10 mg/kg through intraperitoneal route was given. PTZ seizure test was done after 24 h after repeating