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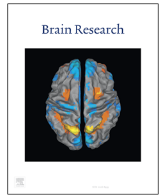

Research report

Impairment of blood brain barrier is related with the neuroinflammation induced peripheral immune status in intracerebroventricular colchicine injected rats: An experimental study with mannitol

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ABSTRACT

The neurodegeneration in AD patients may be associated with changes of peripheral immune responses. Some peripheral immune responses are altered due to neuroinflammation in colchicine induced AD (cAD) rats. The leaky blood brain barrier (BBB) in cAD-rats may be involved in inducing peripheral inflammation, though there is no report in this regard. Therefore, the present study was designed to investigate the role of BBB in cADrats by altering the BBB in a time dependent manner with injection (i.v.) of mannitol (BBB opener). The inflammatory markers in the brain and serum along with the peripheral immune responses were measured after 30 and 60 min of mannitol injection in cAD rats. The results showed higher inflammatory markers in the hippocampus and serum along with alterations in peripheral immune parameters in cAD rats. Although the hippocampal inflammatory markers did not further change after mannitol injection in cAD rats, the serum inflammatory markers and peripheral immune responses were altered and these changes were greater after 60 min than that of 30 min of mannitol injection. The present study shows that the peripheral immune responses in cAD rats after 30 and 60 min of mannitol injection are related to magnitude of impairment of BBB in these conditions. It can be concluded from this study that impairment of BBB in cAD rats is related to the changes of peripheral immune responses observed in that condition.

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1. Introduction

Alzheimer Disease (AD) is a progressive neurodegenerative disease and is characterized by dementia and personality changes (Huberman et al., 1999; Jellinger and Bancher, 1996). The mechanism of neurodegeneration in AD has not been understood clearly. Neuroinflammation has been proposed as one of the important causes of neurodegeneration in AD (de Vries et al., 1997). The inflammatory markers of brain such as ROS, nitrite, TNF α, IL 1β were found to be increased in AD patients (Degena et al., 2007; Alvarez et al., 2007). These inflammatory markers may pass through BBB to the systemic circulation and may have a chance to influence the peripheral immune responses (Britschgi and Wyss-Coray, 2007). It was reported that the monocytes of AD patients adopt proinflammatory status and secrete higher level of IL 6 after stimulation with mitogen phytohemagglutinin (Shalit et al., 1994) and the cytotoxic activity of NK cells was increased in senile dementia of AD type (Soleret et al., 1998). Among the probable causes of immune changes in the periphery of AD patients a leaky BBB may be important as the BBB appeared to be impaired in AD patients and its permeability was increased in that condition (Bowman et al., 2007, Zipser et al., 2007). There is a probability of greater entry of inflammatory markers from brain to systemic circulation in that altered state of BBB in neuroinflammation (de Vries et al., 1997) as a bi - directional communication between brain and periphery has been proposed (Nicola et al., 2013).

Intracerebroventricular colchicine injected rats have been considered by several investigators as an animal model of AD (Shigematsu and McGeer, 1992; Kumar et al., 2007; Ganguly and Guha, 2008; Pitchaimani et al., 2012; Sil et al., 2014; Sil and Ghosh, 2016). Several authors have proposed the neuroinflammation as one of the causative factors for neurodegeneration in different chemically induced animal models of AD, e.g, streptozotocin, okadaic acid and colchicine induced AD rats (Nazem et al., 2015; Sil et al., 2014). The neuroinflammation in i.c.v. colchicine injected AD (cAD) rats was indicated by higher level of inflammatory

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markers (TNF α, IL 1β, ROS and nitrite) in the brain (Sil et al., 2014, Sil and Ghosh, 2016) and these inflammatory markers in the systemic circulation were also increased in cAD rats (Sil et al., 2014). Even these inflammatory markers may influence some of the peripheral immune responses such as increased phagocytic activity of blood WBC/ splenic polymorphonuclear cells, increased cytotoxicity of splenic mononuclear cells and decreased adhesion of mononuclear cells in cAD rats (Sil et al., 2014). If neuroinflammation in the brain of cAD rats is the cause behind the peripheral immune responses in that experimental condition, the efflux of inflammatory markers from brain through a leaky BBB is a great possibility. In support of this contention it may be noted that the fold of increase of inflammatory mediators in the brain of cAD rats were much more than that of periphery in our previous studies (Sil et al., 2014; Sil and Ghosh, 2016).

Mannitol is well-known for its ability to increase the permeability of blood brain barrier (Brown et al., 2004; Chi et al., 1996; Rapoport, 2000). Osmotic opening of the blood brain barrier (BBB) by intracarotid infusion of a hypertonic mannitol solution is mediated by vasodilation and shrinkage of cerebrovascular endothelial cells, with widening of the interendothelial tight junctions to an estimated radius of 20 nm in humans (Rapoport, 2000). Ongoing multicenter clinical studies suggest that BBB disruption by intraarterial hyperosmotic mannitol can enhance the penetration of anticancer drugs and prolong the survival of patients with malignant brain tumours (Muldoon et al., 2007). Mannitol has been used in several rat models for increasing the permeability of BBB (Brown et al., 2004; Paczynski et al., 1997; Lin et al., 1997; Joshi et al., 2011). The greater passage of radioactive rubidium, potassium and sucrose from blood to brain was observed in rats after intracarotid mannitol injection (Brown et al., 2004). Other investigators have reported decreased brain edema induced by hypoxic ischemia after intravenous (Paczynski et al., 1997) or subcutaneous injection of mannitol (Mujsce et al., 1990). Mannitol induces increase of BBB permeability in a time dependent manner in rats (Lin et al., 1997; Joshi et al., 2011). It was found that the permeability of BBB was gradually increased reaching a peak at about 1 h after injection of mannitol (Joshi et al., 2011).

The hypothesis of impairment of blood brain barrier in the cAD model can be verified by increasing the permeability of BBB in cAD rats experimentally (e.g. i.v. injection of mannitol) and by measuring the inflammatory status in brain and periphery in different time windows of that experimental condition. The role of BBB, if any, in the passage of inflammatory markers from brain to periphery in cAD rats may be assessed by comparing the levels of inflammatory markers in periphery of cAD rats and mannitol treated cAD rats in different time points. However, there is no report in literature in this regard. Therefore, the present study was designed to investigate the role of blood brain barrier in changing the peripheral immune responses in cAD rats by experimentally altering the BBB in a time dependent manner with the injection (i.v.) of mannitol. The inflammatory mediators in the brain and serum along with the peripheral immune responses were measured after 30 and 60 min of mannitol injection in cAD rats to assess the inflammatory status in the brain and periphery of these animals in a time dependent manner and to corroborate these changes with the observed immune responses in two time points. Thus, an attempt has been made to identify the importance of BBB in eliciting the peripheral inflammatory state in cAD rats.

2. Experimental procedures

2.1. Animals

Charles-Foster rats (male, 200–250 g, 6–8-wk-of-age) and Swiss albino mice (male, 20–30 g, 6–8-wk-of-age; for use in passaging of Ehrlich ascites cells in one study outlined below) were obtained from local animal supplier (M/s Chakraborty Enterprise, Kolkata, India) for use in this study. All animals were housed individually in polypropylene cages in a facility maintained at 25 ± 1 °C with a 12-h light dark cycle. All animals had ad libitum access to standard rodent chow food pellets and filtered water. The University of Calcutta Animal Ethics Committee approved all protocols used in these studies.

2.2. Experimental design

2.2.1. Experiment I

Rats were divided into three groups: control rat, sham-operated rats (receiving only intra-cerebroventricular [i.c.v.] injection of artificial CSF (Sil et al., 2014), and AD rats (receiving i.c.v. colchicine). On Day 21 after the i.c.v. injection of colchicine or artificial CSF, all rats (including controls) were anaesthesized with ether, and the spleens of subsets of three rats in each group (i.e. 9 rats/group which gave 3 observations) were pooled to get the requisite number of splenic cells to measure leukocyte adhesive inhibition indices (LAI), phagocytosis by polymorphonuclear (PMN) cells and the cytotoxic activity of splenic mononuclear cells (MNC). The phagocytic activity of white blood cells (WBC), levels of TNFα, IL 1β, ROS, and nitrite in the hippocampus and serum were also assessed in 6 rats of each group.

2.2.2. Experiment II

Rats were divided into three groups: control rat, sham-operated rat and cAD rats. On Day 21 after the i.c.v. injection of colchicine or artificial CSF, all rats (including controls) were anaesthesized with ether and all the rats from each group received 1 M i.v. mannitol injection and sacrificed after 30 min. The various parameters as noted in Experiment I were also measured here, following the same experimental design in all the groups of rats.

2.2.3. Experiment III

Rats were divided into three groups: control rat, sham-operated rat and cAD rats. On Day 21 after the i.c.v. injection of colchicine or artificial CSF, all rats (including controls) were anaesthesized with ether and all the rats from each group received 1 M i.v. mannitol injection and sacrificed after 60 min. The various parameters as noted in Experiment I were also measured here, following the same experimental design in all the groups of rats.

2.3. Intracerebroventricular injection of colchicine in rats

Colchicine (7.5 µg in 2.5 µl of artificial CSF) was injected into the lateral ventricle of each side of the rat brain using stereotaxic coordinates: AP: −0.6 mm from bregma, L. ± 1.5 mm from midline and V:2.8 mm from the skull surface (Paxinos and Watson, 1986). Rats were anaesthetized with Na-thiopentone (50 mg/kg BW), and the head of the rat was fixed on the stereotaxic apparatus (ST141, INCO Ambala, Delhi, India) with the help of ear bars, incisor bar and nose clip. The skin covering the skull was opened by midline incision and periosteum over the skull surface was retracted. Burr holes were made on the two points over the skull surface (right and left side) according to the AP and L coordinates, with a dental drill. A steel micro-cannula (0.45 mm diameter) connected to a 10 µl Hamilton syringe (Hamilton, Australia) with polyethylene tubing, was inserted into the lateral ventricle using the V coordinate. The cannula was left in place for 2–3 min after the i.c.v. injection. Sham-operated rats received the same volume (2.5 µl) of artificial CSF in each lateral ventricle by the same procedure. The trephine hole was covered with sterile bone wax after withdrawal of injecting needle. The muscles and skin were then sutured

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