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Effects of lesion of posterior part of cerebellar vermis and fastigial nuclei on some immune responses in male rats

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Abstract

Considering the autonomic function of cerebellum, it is a potential neural area for immunomodulation. Though the role of fastigial nucleus (FN) and vestibulocerebellum on immunomodulation has been investigated, the results are contradictory. In the present study the role of posterior cerbellar vermis (PV) and FN on some immunological parameters such as TC and DC of WBC, leukocyte adhesive inhibition index (LAI) of splenic mononuclear cell (MNC) and phagocytic activity of peripheral WBC by fluorescence activated cell sorter (FACS) were investigated after two and three weeks lesions of PV (lobules VII, IX and X) and FN by aspiration technique in male rats. The TC of WBC was decreased after two weeks of PV+FN lesioned rats compared to that of sham lesion and control rats. The percentage neutrophil and eoisinophil counts were increased and the lymphocyte count was decreased after two weeks of PV+ FN lesioned rats compared to that of sham operated and control rats. The phagocytic activity of blood WBC and LAI of splenic MNC were increased after two weeks of PV+FN lesioned rats compared to that of sham lesioned and control rats. After three weeks of PV+FN lesioned these immunological parameters were regain to the normal level. The results indicate that the combined lesion of PV and FN induced enhanced immune responses but this effect is transitory.

Key words: Cerebellum, Immune responses, Phagocytic activity, LAI

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INTRODUCTION

The recent advancement in the field of neuroimmunomodulation has shown that the nervous system and the immune system are closely inter-linked [1, 2] and the role of different brain areas on immune responses has been reported by several investigators [3-9]. Several reports indicate that the different brain areas like hypothalamus, amygdala, hippocampus, stria terminalis, and septal nuclei play an important role in neuroimmunomodulation [3-6, 9, 10, 12, 13].

The cerebellum is a part of the brain that serves as a regulator of voluntary motor activity, relay station for unconscious proprioception, and balance [14, 15]. Besides its well-known role on in voluntary motor activities, cerebellum has been found to be involved with regulation of the activities of the cardiovascular, respiratory, gastrointestinal systems [16-23]. There are many reports which indicate that the anterior vermal part of the cerebellum has modulatory influence on the different autonomic functions [15, 24, 25]. The posterior part of the cerebellar vermis is also involved in the regulation of the autonomic functions [20, 26]. The autonomic functions are also regulated by the vestibulocerebellum [27]. The cerebellar vermis including its posterior part exerts its influence through the output to the fastigial nuclei (FN) [28]. The electrical stimulation of FN predominantly elevates respiratory frequency associated with the pressor response [29, 30].

The neural areas of brain regulating the activity of autonomic nervous system are potential candidates as a centre of immunomodulation because neural areas regulate immune system by sympathetic activity and hypothalamo-pituitary-adrenal (HPA) axis. Considering the autonomic role of cerebellar vermis and FN, it appears this portion of cerebellum may be involved with immunomodulation. However, only few investigators have explored the immunomodulatory activity of cerebellum [31-33]

Ghoshal et al [31] reported that the kainate induced lesion of the vestibulocerebellum reduced the peripheral blood leukocyte concentration, neutrophil myeloperoxidase response, T-SRBC rosette and antibody titer to sheep red blood cell (SRBC). Peng et al [32] observed some immune responses after lesion of FN with kainite and reported that Con A induced lymphocyte proliferation and NKCC were significantly enhanced in rats. Apparently the immunostimulatory effects of FN lesioned rats are opposite to the immunosuppressive effects observed in vestibulocerbellar lesioned rats [31]. The cerebellar vermis usually inhibits FN by GABAergic Purkinjee axons and the opposite immunomodulatory role of FN and vestibulocerebellum may be explained on the basis of this inhibitory regulation of FN by vestibulocerebellum. However, the leukocyte migration inhibition was increased with decrease in DTH in electrolytic lesion of FN in male rats [33].

The vestibulocerebellum has direct connection with vestibular nucleus bi-directionally in addition to its connection with FN [34]. In FN lesioned rats this neural connection may be intact and may take part in the regulation of immune system. A combined lesion of nodule, uvula (components of PV) and FN will eliminate all such connections and may be helpful to explore the immunomodulatory functions of cerebellum. The present study has been carried out to assess immunomodulatory role of lobule VII, IX, X of cerbellar vermis and FN by combined ablation of these areas of rat by aspiration method

METHODS

Animals:

In this study healthy, adult (6-8 weeks of age) male albino rats (Charles-Foster strain) weighing 200-220 g were used. Animals were housed individually in polypropylene animal cages with food pellet and water ad libitum in the animal room with a 12h light dark cycle (light 7 AM to 7 PM). Animal room was maintained at the temperature of 25 ± 1oC. According to Institutional Animal Ethical Committee all adequate measures were taken to minimize the pain and discomfort to the rats.

Design of experiments:

Experiment – I [Phagocytic activity of blood WBC and hematological parameters]:

Thirty six rats were equally divided into three groups: control, sham operated and combined lesion of posterior part of vermis and fastigial nuclei (PV+FN). The phagocytic activity of blood WBC, total count (TC) of WBC and differential count (DC) of WBC were measured in 6 animals of each groups after two weeks of surgery. These parameters were also measured in rest of the 6 rats of each group after three weeks after surgery.

Experiment – II [Leukocyte Adhesive Inhibition Index (LAI)]:

(a) Study after two weeks of lesion: Twenty seven rats were equally divided into three groups as in experiment-II. Nine rats in each group were divided into 3 sub-groups (3 rats in each sub-group). The LAI was measured two weeks after surgery in PV+FN lesioned and sham operated groups and also in control group from pooled spleen of 4 animals in each sub-group as number of mononuclear cells obtained from one spleen was not sufficient for measurement of LAI. Thus three sets of data were obtained from each group.

(b)Study after three weeks of lesion: Twenty seven rats were equally divided into three groups as in experiment-II for the measurement of LAI after 3

weeks of surgery. Three animals were used as a sub-group to measure the LAI like that in the study after two weeks of lesion.

Surgery:

The head were fixed horizontally in the stereotaxic apparatus. Then the posterior part of occipital bone was exposed by retraction of nuchal muscles and a trephine hole was made on middle and posterior end of the occipital bone. The posterior lobe of cerebellum (lobules VII, IX and X) and FN was aspirated with vacuum pump as described by Mayers [35]. Na-thiopentone (50 mg/kg body wt, i.p.) was used as anesthesia.

Blood collection:

The blood was collected (0.5 ml) from the heart of a deeply anesthetized rat (Na- thiopentone, 50 mg/kg body wt, i.p.) by a syringe containing 100 µL of Na-citrate (3.8%, Sigma) between 2:30 to 3:00 PM on the day of sacrificing the rats (after 2 weeks or 3 weeks of surgery) for Fluorescence Activated Cell Sorting (FACS) analysis. 1 ml of blood was also collected and mixed with 0.1g ethylene diamine tetra-acetic acid (EDTA) for the determination of TC and DC of WBC.

TC of WBC and DC of WBC

TC of WBC was determined using Neubaur haemocytometer and DC of WBC was determined microscopically on the blood film after staining with Leishman's stain (Merck, India) [38].

Phagocytic Activity of Blood WBC by FACS Analysis

According the method of Oben and Foreman [36] the Fluorescein isothiocyanate (FITC) (Sigma, USA) tagged bacterial cell membrane was prepared. 100 µl of blood sample was taken from the collected blood in four micro centrifuge tubes. 20µl of FITC tagged bacterial cell membrane and 380µl of Roswell Park Memorial Institute (RPMI) medium (AT1640, HIMEDIA, India) were mixed in each of the four micro centrifuge tubes and tubes were incubated for different time durations such as 0, 15, 60 and 90 minutes at 37oC. After incubation the tubes were dipped into ice for 15 minutes to stop the reaction. Then 1 ml of RBC lysing solution (10 times dilution with distilled water) (BD, USA) was added in each tube and the tube was left for 5 minutes in dark. The tube was centrifuged at 800 g for 3 minutes and was washed with phosphate buffer saline (PBS, pH 7.4). Mean Fluorescence values were analyzed by Becton Dickinson FACS caliber using Cell-Quest Software after 5000 cell count. Mean fluorescence values of samples for different time duration studies were taken from FITC positive cell population from histogram. A regression line was drawn from the mean fluorescence values of different time intervals (MINITAB statistical software) for each group of rats. The slope of the regression line was considered as phagocytic Index (PI) of blood WBC.

Leukocyte adhesive inhibition index (LAI)

The spleens of rats were collected aseptically after deep anesthesia (Na- thiopentone, 50 mg/kg body wt, i.p.) and the leukocyte adhesive inhibition index (LAI) was measured according to the methods of Maluish et al [37]. The spleens of 3 rats of a sub-group were put together in PBS containing 3.8% Na-citrate (Sigma, USA) in a ratio of 10:1 (v/v). Single cell suspension of those spleens was made and mononuclear cells (MNC) were separated by percoll density gradient 1.077 densities. MNC (98% purity of separation) count in isolated suspension was taken by Neubaur haemocytometer and the haemocytometer was incubated for 30 minutes at 37ºC in a moist environment. After 30 minutes of incubation the counted field was washed gently with PBS by Pasteur pipette and adhered cells were counted. The Percentage of LAI = (No. of adhered cell after incubation x 100) / (No. of total cell count before incubation). A smear of the isolated MNC suspension was made and was stained with Leishman's stain (Merck, India). The percentage of mononuclear cells in this smear was determined to verify the purity of separation.

Confirmation of lesion by histology:

The animals were sacrificed at the end of experiment and were perfused intracardially with 0.9% saline followed by 10% formaldehyde solution. The brains were removed from the skulls and were kept in 10% formalin solution for fixation. After dehydration and clearing, paraffin blocks of those brains were prepared and 10µm thick sections were cut by microtome. The brain sections were stained by haematoxylin-eosin to identify the lesioned area. (Fig 2)

Statistical analysis:

Data are expressed as mean ± SEM. One-way ANOVA was employed to compare the data of the control, sham operated and cerebellar PV lesioned groups followed by LSD post hoc test using the statistical package for social science software (SPSS software: 9.0.0, USA). $P \leq 0.05$ was considered as a significant difference.

RESULTS

Experiment I

Phagocytic Activity of Blood WBC by FACS Analysis

The PI in the posterior part of vermis and fastigial nuclei (PV+FN) lesioned group (2 weeks after surgery) was significantly decreased compared to that in the control [$F(4, 25) = 42.549$, $p \geq 0.001$] and sham-operated groups [$F(4, 25) = 42.549$, $p \geq 0.001$] (Fig 3). The PI in PV+FN lesioned rats 3 weeks after surgery did not show any significant change compared to that in the control and sham-operated rats.

Conditions	TC of WBC / µl of blood	DC of WBC (%)				
		Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	8741.7 ± 556.89	22.8 ± 1.01	72.7 ± 0.795	3.2 ± 0.479	1.1 ± 0.211	0.5 ± 0.224
2 weeks after sham operation	9308.3 ± 855.21	21.5 ± 0.849	74.2 ± 0.949	2.8 ± 0.479	1.2 ± 0.544	0.3 ± 0.212
2 weeks after PV+FN lesion	6266.7 ± 475.42*^	32.3 ± 1.48*^	59.8 ± 0.984*^	4.3 ± 0.559	3.2 ± 0.705*^	0.3 ± 0.334
3weeks after sham operation	9300.0 ± 923.21	22.5 ± 0.766	73.2 ± 0.984	2.7 ± 0.423	1.2 ± 0.544	0.5 ± 0.342
3 weeks after PV+FN lesion	7266.3 ± 710.52	25.6 ± 1.14	68.5 ± 1.38	3.7 ± 0.669	2.0 ± 0.367	0.2 ± 0.167

Table 1 Total count (TC) and Differential count (DC) of WBC in PV+FN lesioned, sham operated and control rats 2 weeks and 3 weeks after surgery with the TC and DC of WBC in control rats. The TC of WBC and the percentage of lymphocyte were significantly decreased in PV+FN lesioned rat and the percentage of neutrophil and eosinophil was significantly increased in PV+FN lesioned rat compared to control and sham operated rats 2 weeks after surgery.
Values are expressed as mean ± SEM.(n=6 in each group) *p<0.01 for significant difference between control rats and 2 weeks after PV+FN lesioned rats; ^p<0.05 for significant difference between 2 weeks after PV+FN lesioned rats and 2 weeks after sham operated rats.

TC and DC of WBC

The TC of WBC was decreased in PV+FN lesioned rats 2 weeks after surgery compared to that in the control [F (4, 25) = 3.531, p ≥ 0.01] and sham-operated groups [F (4, 25) = 16.636, p ≥ 0.001]. The percentage of neutrophil was increased [F (4, 25) = 16.636, p ≥ 0.001], lymphocyte was decreased [F (4, 25) = 31.886, p ≥ 0.001] and eosinophil was increased [F (4, 25) = 3.183, p ≥ 0.01] significantly in the PV+FN lesioned group (2 weeks after surgery) compared to that in the control and sham operated group (Table 1). The TC and DC of WBC remain unaltered in PV+FN lesioned group after 3 weeks of surgery compared to that in the control and sham operated group

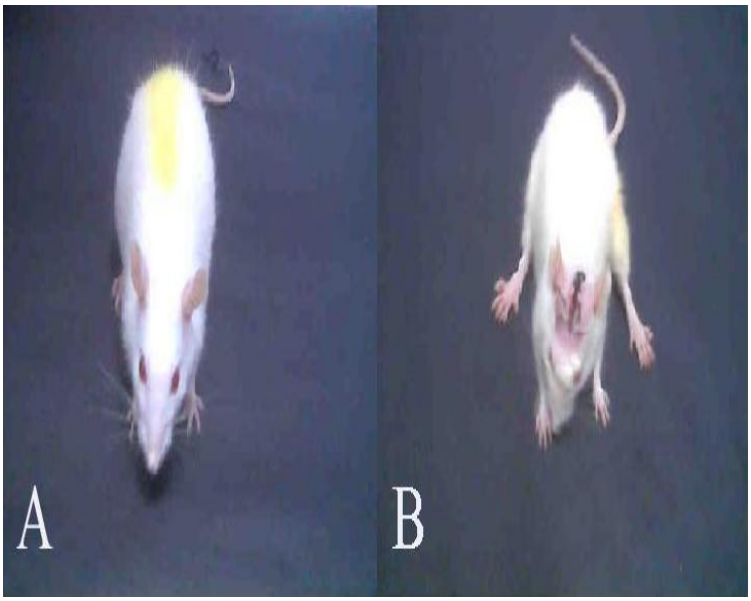

Fig 1 The picture of Control rat (A) and after combined lesion of posterior part of vermis and fastigial nuclei (PV+FN) (B).

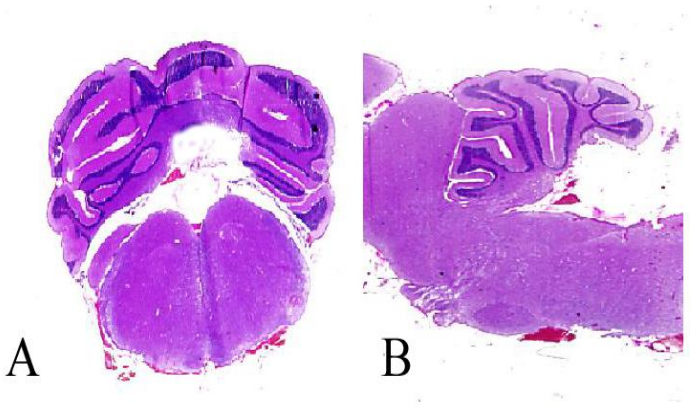

Fig 2 Haematoxylin and eosine stained coronal (A) and sagittal (B) section of rat brain showing the combined lesion of posterior part of vermis and fastigial nuclei (PV+FN) area.

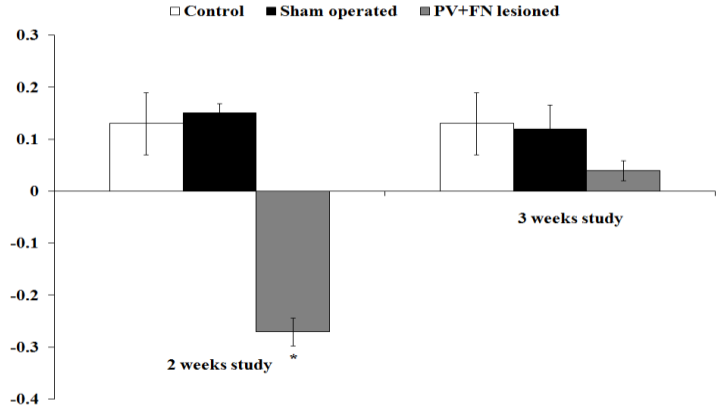

Fig 3 The phagocytic index (PI) in combined lesion of posterior part of vermis and fastigial nuclei (PV+FN), sham operated and control rats two weeks and three weeks after surgery. The phagocytic index (PI) in PV+FN lesioned rats after two weeks of surgery was decreased (*p < 0.01) compared to control and sham operated rats. The phagocytic index in PV+FN lesioned rats after three weeks of surgery was not changed significantly compared to control and sham operated rats. Values are expressed in mean ± SEM. (n=6 in each group).

Experiment II

Percentage of LAI

The percentage of LAI was significantly increased in the PV+FN lesioned group (2 weeks after surgery) compared to that in the control [F (4, 25) = 3.828, p ≥ 0.01] and sham-operated groups [F (4, 25) = 3.282, p ≥ 0.01]. The percentage of LAI remained unaltered in the PV+FN lesioned group (3 weeks after surgery) compared to that in the control and sham-operated groups (Fig 4).

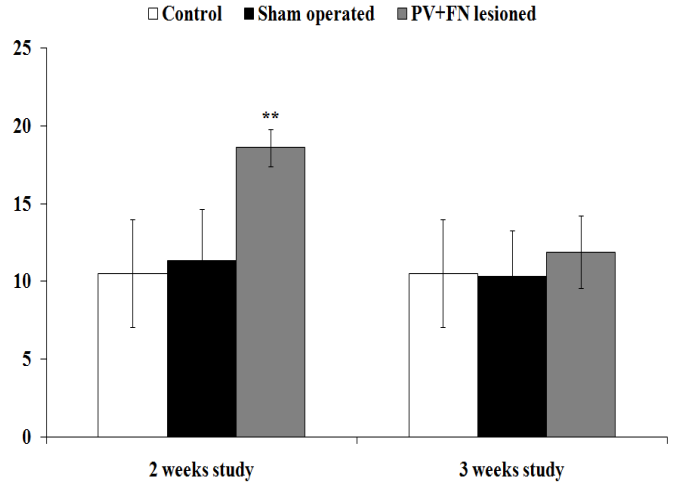

Fig 4 Leukocyte Adhesive inhibition Index (LAI) in combined lesion of posterior part of vermis and fastigial nuclei (PV+FN), sham operated and control rats two weeks and three weeks after surgery. The percentage of LAI in PV+FN lesioned rats two weeks after surgery was significantly increased (**p<0.001) compared to control and sham operated rats.The percentage of LAI in PV+FN lesioned rats was not significantly changed 3 weeks after surgery compared to control and sham operated rats. The values of LAI are expressed as mean (mean values of 3 sub-groups) ± SEM. (each sub-groups provides pooled values of 4 animals).

DISCUSSION

In the present study the TC of WBC was decreased two weeks after combined lesion of posterior vermis and fastigial nuclei (PV+FN) in rats. The percent of neutrophil was significantly increased whereas the percentage of lymphocyte and eosinophil were decreased in rats after two weeks of PV+FN lesion. It is known that the peripheral blood lymphocytes originated from different immune organs and they maintain the homeostasis to perform the normal immune tasks. The reduction of the percentage of lymphocyte induced by PV+FN lesion indicates the modulatory role of PV+FN on immune functions. Ghoshal et al [31] was also reported that the TC of WBC and percentage of lymphocyte was decreased and the percentage of neutrophil and eosinophil were increased 45 days after the kainite induced lesion of vestibulocerebellum. Though there are no reports on the changes of DC of WBC after lesion of FN, this study shows that the percent changes of WBC in the PV+FN lesioned rats are similar to that of vestibulocerebellum lesioned rats [31]. However, it was noted that the observed changes of percent of WBC almost returned to control level after three weeks of PV+FN lesion.

The phagocytic activity of blood WBCs (neutrophils, eosinophils and monocytes) was increased two weeks after PV+FN lesion. The increased phagocytic activity of blood WBC is probably due to the increased activity of neutrophils, as the percent of neutrophils was increased significantly in PV+FN lesioned rats after two weeks of surgery. The phagocytic activity has not been measured in peripheral blood after experimental manipulation of cerebellum in rats by others authors. The present study indicates that a stimulatory effect on phagocytic activity of peripheral blood and adhesibility (LAI) of splenic mononuclear cell (MNC) in rats after combined lesion of PV and FN (PV+FN). Though the enhanced Con A induced lymphocyte proliferation and natural killer cell cytotoxicity (NKCC) were noted in FN lesioned rats from 8-32 days after surgery by Peng et al [32], the present study shows the phagocytic activity of peripheral blood WBC and LAI of splenic MNC returned to the normal level after three weeks of surgery. This recovery of immune function may be due to the combined lesion of PV and FN. Moreover this recovery indicates that the observed changes are not due to the general inflammatory responses.

The FN lesioned induced changes have been explained by Peng et al [32] on the basis of alteration of sympathetic activity in absence of FN which might influence the hypothalamus by direct cerebello-hypothalamic projection [21, 22]. Peng et al [32] did not consider hypothalamo-pituitary-adrenal (HPA) axis as important regulator of immune changes in their experiment. Gajalakshmi et al [33] noted significant alteration of IL2, IL4 and IFN gamma in FN lesioned rats. As the corticosterone and cytokines were not measured in the present study; the mechanism of the observed changes cannot be identified.

CONCLUSION

The present study indicate that the combined lesion of PV and FN (PV+FN) induced enhanced immune responses but this effect is transitory

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