EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF NGF ON BLOOD BRAIN BARRIER AND STRESS INDUCED PROTEIN IN THE CENTRAL NERVOUS SYSTEM

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SUMMARY: Nerve growth factor (NGF) is the first member characterized of the neurotrophin family. It is known for its crucial role in survival, differentiation and maintenance of neurons both in peripheral and central nervous systems. In addition to its neurotropic role, NGF has also been proposed to act on cells of the immune system. Recent studies show that there is an increased level of NGF in cerebrospinal fluid (CSF) during the acute phase of multiple sclerosis, in animal model of multiple sclerosis and experimental allergic encephalomyelitis (EAE). In contrast, during the remission phase of the diseases the levels of NGF drop significantly. More recently, the increased level of NGF has also been reported in other autoimmune diseases such as lupus erythematosus. These observations suggest that over production of NGF in CNS may functionally be related to the state of activation of the immune system in autoimmune diseases. Concomitantly, proinflammatory cytokines are upregulated in the acute phase of autoimmune diseases and are known to be potent inducers of the expression of heat shock proteins (HSP). Moreover, NGF is known to be a chemotactic factor for polymorphonuclear cells (14, 15). Due to concomitantly increased level of NGF in inflammatory sites and around the blood vessels in acute phase of the disease with leukocyte infiltration of the immune cells in CNS, one may question whether NGF has any effect on production of proinflammatory cytokines through production of heat shock proteins and leukocyte infiltration. To answer the above questions, NGF was injected intracerebroventricularly at doses 20 or 5 μg/mice for 4 days. The results show that the administered NGF neither has any effect on the expression of HSP-27 nor on leukocyte infiltration in central nervous system, suggesting that the high doses of NGF utilized in these experiments affect neither the immune nor the central nervous systems.

Key Words: Blood brain barrier, intracerebroventricular, NGF, HSP.

INTRODUCTION

Nerve growth factor (NGF), the first identified neurotrophin, has various effects on growth, proliferation and differentiation of neural crest derived cells and sympathetic neurons (2–5, 7). NGF also interferes with the immune system and it is therefore conceivable that any disturbances in its activity or synthesis may interrupt functions in both nervous and immune systems (6–8).

Otten and colleagues (18) have reported an increased level of NGF in cerebrospinal fluid of multiple sclerosis patients during disease attacks and conversely a reduction during the remissions. Moreover, the level is also increased in EAE, animal model of multiple sclerosis and in autoimmune diseases such as lupus erythematosus

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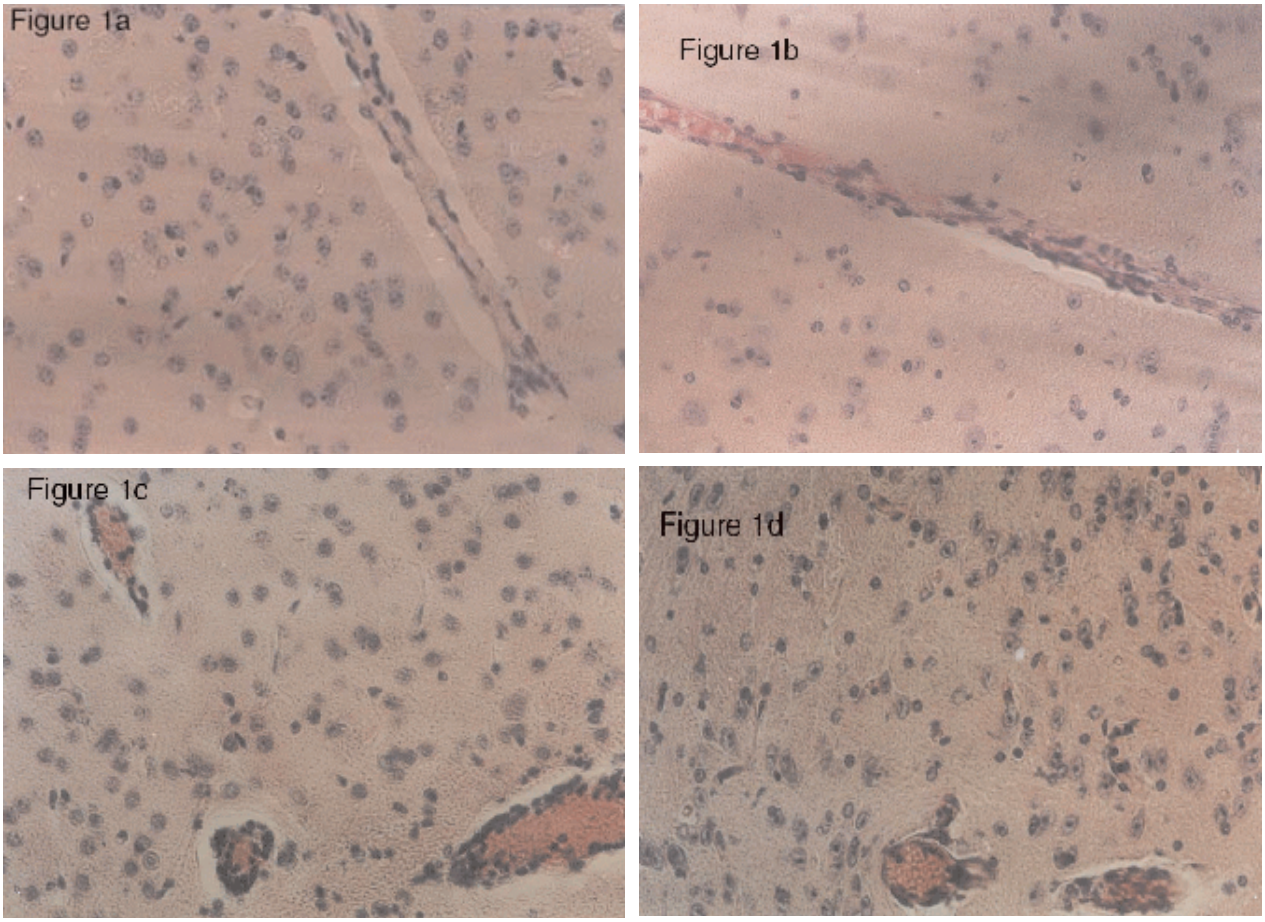
Figure 1 : Photomicrographs of H and E staining of brain parenchyme in untreated (a, c) and NGF treated (b, d; 5 μg/mice). Arrows point to the sections from brain capillaries with no signs of cell infiltration either in treated or untreated mice.



and rheumatoid arthritis (10–13). This increase in NGF during the acute phase of the disease suggests a presumptive role of NGF in the disease.

In vitro and *in vivo* experiments have indicated NGF as a chemotactic factor for polymorphonuclear cells (14, 15). Due to concomitantly increased level of NGF in inflammatory sites and around the blood vessels in acute phase of the disease and leukocyte infiltration of the immune cells in CNS, one may question whether NGF has any effect on immune cell infiltration.

Evidence have also shown that in autoimmune diseases such as multiple sclerosis, there is an increased level of inflammatory cytokines (18–20) which in turn may affect other factors such as heat shock proteins (17). To indirectly uncover the possible effect of NGF on the synthesis of cytokines, changes in the level of heat shock proteins (HSP) should be considered. In the present study therefore, the possible effects of an increased level of NGF on immune cell infiltration and on HSP-27 has been investigated.

MATERIALS AND METHODS

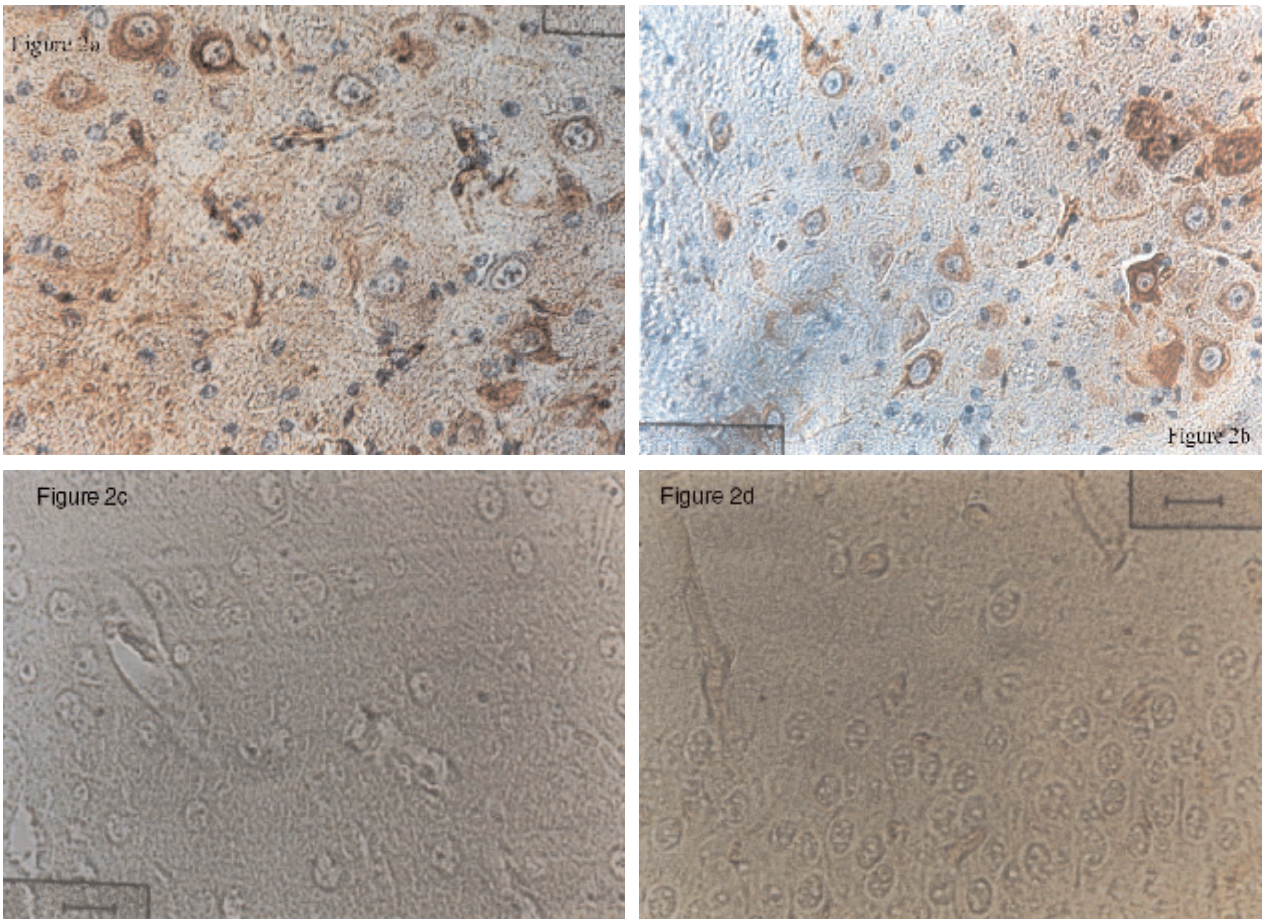
Animals and treatment

Six-eight week old SJL/J female mice (Charles River Company) were purchased and a period of one or two weeks of acclimatization allowed. To inject NGF intracerebroventricularly, a cannula was placed in the right ventricle stereotactically. Following one week recovery, NGF was injected at 5 or 20 μg/mice. Control group received a solution of phosphate buffered saline (PBS) instead.

Histology and immunohistochemistry

Brains were placed in Bouin's fixative, paraffin blocked, sectioned and stained using H and E staining. Sections were then investigated for signs of cell infiltration. Moreover, paraffin slides were dewaxed and processed for immunohistochemistry. Briefly,

Figure 2 : Photomicrographs of HSP-27 immunostaining in brain stem and striatum of untreated and NGF treated mice. Brain stem in untreated mice (a) and NGF treated mice (b; 20 μg/mice). Striatum in untreated mice (d) and NGF treated mice (c; 20 μg/mice). Positive HSP immunoreactive neurons can be seen in brain stem, but not striatum of treated and untreated mice with no difference in terms of intensities.



slides were incubated in H_2O_2 (3%, 10 mins), washed with PBS-Triton X-100 (5 times, 3 mins), blocked with blocking buffer (5% normal rabbit serum+0.8% BSA+0.25% Triton X-100, 30 mins, 37ºC). They were then incubated in primary antibody (Goat anti-IgG, HSP-27, 1/100, 2 hrs, 37ºC; Santa Cruz), washed in PBS-Triton X-100 (5 times, 3 mins) followed by an incubation in secondary antibody (rabbit biotinylated anti goat IgG, 1/250, 2 hrs, RT; Sigma). Slides were then washed in PBS-Triton X-100 (5 times, 3 mins) and incubated in ABC solution (Avidin-biotin-horseradish peroxidase, 1/100, 1 hr; DAKO). Finally after washes in PBS-Triton X-100 (5 times, 3 mins), the reaction was developed by DAB (Diaminobenzidine tetrahydrochloride, DAKO, 2 mins). Development was terminated by washes with PBS (5 times, 3 mins) and counterstaining was performed using hematoxilin. Slides were then dehydrated and mounted.

RESULTS

The results in Figures 1a-1d show that no leukocyte infiltration in periphery of the vessels can be seen in mice treated with NGF (5, 20 μg/mice) in comparison with mice treated with PBS, suggesting that NGF treatment has not affected on immune cell infiltration.

The results in Figures 2a-2b show that there is no difference in the intensity of HSP-immunoreactivity in brain stem of NGF treated mice in comparison with that in PBS treated mice. Moreover, in other areas of the brain such as striatum with no positive HSP immunoreactivities under normal circumstances, no induction of HSP immunoreactivity can also be seen in NGF treated mice in comparison with PBS treated mice (Figures 2c-2d). Altogether, these results indicate that NGF treatment does not change the HSP level in brain.

DISCUSSION

In this study, we sought to determine if overload of NGF in the central nervous system induces the immune response which may in turn affect the nervous system. According to these results, administered NGF at the dose and for the selected duration was unable to induce an inflammatory response which may activate the endothelial cells. It would therefore be concluded that overloading NGF prevents the breakdown of the blood brain barrier (BBB) which would in turn make cellular invasion possible.

Furthermore, the results from immunohistochemical studies show that NGF has no effect on HSP expression either in brain stem or other areas of the brain. This may indicate that NGF overload alone is possibly not a stress inducing factor for the nerve cells. Taken together from the above findings, we suggest that NGF alone is not a stress-induced agent and may require additional agents to act so.

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