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ORIGINAL ARTICLE

Infliximab administration reduces neuronal apoptosis on the optic pathways in a rabbit hydrocephalus Model: A preliminary report

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Abstract

Object. This study was designed to explore the effects of infliximab on the optic pathway in kaolin induced hydrocephalus rabbit model. *Methods.* After injection of kaolin to the cisterna magna of 12 New Zealand rabbits for induction of hydrocephalus, animals were divided into 2 groups and received either infliximab or normal saline. The intracranial pressure measurement was performed 2 times; firstly, before kaolin injection and secondly, before decapitation to ensure that the rabbits had hydrocephalus. After 2 weeks, animals were decapitated. *Results.* Apoptotic cells in the lateral geniculate body, optic radiation, and optic disc were counted with TUNEL method. Apoptotic cell counts of the lateral geniculate body and the optic radiation were showed statistically significant difference between the infliximab group and the control group. *Conclusions.* This study suggests that infliximab may have a neuroprotective effect through its anti-apoptotic property on hydrocephalus induced optic pathways injury.

Key words: *Hydrocephalus, apoptosis, tumor necrosis factor alpha, infliximab.*

Introduction

Intracranial pressure raising (ICP), which is commonly associated with traumatic brain injury and hydrocephalus, can have a deleterious impact on central nervous system structure and function.[1] Hydrocephalus is a common neurological condition characterized by pathologic dilation of the cerebral ventricles. It is usually caused by obstruction of cerebrospinal fluid (CSF) flow. The pathophysiology of hydrocephalus induced brain damage is multifactorial, with contributions made by gradual physical stretching, compression, ischemia and possible accumulation of metabolic waste products.[2,3]

Tumor necrosis factor-α (TNF-α), a pheliotropic cytokine synthesized by a variety of cell types, acts as an important mediator of inflammatory responses.[4] In the brain, both resident macrophages, as well as astrocytes and microglia have the capacity to synthesize this proinflammatory and tissue destructive cytokine.[5,6] TNF-α is toxic for the oligodendrocytes producing myelin, mediates myelin damage *in vitro*,[7] and induces apoptosis of oligodendrocytes.[8,9]

Infliximab is a monoclonal chimeric (mouse-human) immunoglobulin (Ig) G1 that binds to TNF-α.[10] Specifically, infliximab is established as an effective agent in the treatment of rheumatoid arthritis (RA) and Crohn's disease for which conventional therapy has not been effective.[11–14] By blocking the action of TNF-α, infliximab reduces the signs and symptoms of inflammation and improves the condition of patient by reducing disease activity. In addition, infliximab has been established for the treatment of psoriatic arthritis (PsA), ankylosing spondylitis (AS), and Wegener's granulomatosis.[13,15,16]

Kaolin, an inert silica derivative, is a well accepted agent for inducing hydrocephalus in infant and adult animals (mouse, rat, rabbit, hamster, cat, and dog) via injections into the CSF, with no evidence of direct pathological effects on structures distant to the injection site.[17] In kaolin induced hydrocephalus rabbit model, effects of infliximab has not been studied before. The aim of the present study was to examine the CSF levels of TNF- α in a rabbit model of hydrocephalus before and after the infliximab treatment; and, was to assess whether infliximab treatment following hydrocephalic insult in rabbits would reduce optic pathways damage.

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Materials and methods

Animal preparation

The rabbits were provided by the Center of Reproduction of Animals for Experimental Studies of the Gazi University Medical School. The experiment was approved by the local animal use committee. Rabbits were kept under constant laboratory conditions of 18 to 21°C room temperature, a 12-hour light-dark cycle and were allowed free access to food and water. Twelve male, adult New Zealand rabbits weighing 5100 to 6200 g were anesthetized with ketamine/xylazine (50/10 mg/kg intramuscularly). After shaving and flexing the neck, a 23-gauge needle was inserted into the cisterna magna, and 0.1 to 0.2 ml of sterile kaolin suspension (250 mg/ml) was injected slowly. After 24 hours, animals were randomly divided into two groups. In Group I (Control Group) (n = 6) received 5 cc subcutaneous saline. In Group II (Treatment Group) (n = 6) received one dose 5 mg/kg of infliximab subcutaneously. The rabbits were decapitated at the end of the 2 weeks with overdose anesthetic agent. The intracranial pressure measurement was performed 2 times; firstly, before kaolin injection and secondly, before decapitation to ensure that the rabbits had hydrocephalus.

Intracranial pressure

To measure the intracranial pressure (ICP) in rabbits, under anesthetized with ketamine/xylazine (50/10 mg/kg intramuscularly) a 2 mm hole was drilled into the right frontal plate of the skull at a point 5 mm from the midline and 5 mm anterior to the coronal suture. A microsensor probe (0.4 mm diameter; Camino, Deltamed, Ankara) attached to transducer was inserted in the cortical parenchyma and pressure was recorded for 10 min.

Histopathological and biochemical studies

At the time of decapitation, blood samples were collected by cardiac puncture and CSF samples were collected from the cerebellomedullary cistern. Serum and CSF TNF-α concentrations were measured by using sandwich enzyme-linked immunosorbant assay (ELISA) that was based on the cytokine ELISA protocol and expressed as pg/ml (TNF-α Instant Elisa Kit, Bendermed, BMS223 INST, Lot No: 45382067).

Tissues of the optic nerves, the chiasm, both of the lateral geniculate bodies, and anterior part of the optic radiations the parietal were collected bilaterally from brain slices under a stereoscopic microscope. Two sets of specimens were harvested from each rabbit. A total of 24 specimens were evaluated.

Histopathological evaluation was performed by a pathologist blinded to the groups. Lateral geniculate body, optic radiatio and optic disc tissues were fixed in 10% formalin. Tissue sections of 4 μm were cut from representative formalin-fixed and paraffin-embedded tissue blocks. Sections were de-parafinized in xylene and rehydrated. Each sample was stained with hematoxylin-eosin.

Apoptotic cells were detected by TdT-dUTP terminal nick end-labeling (TUNEL) method. Following deparaffinization and inactivation of endogenous peroxidase using 3% hydrogen peroxidase, the slides were incubated with 20 mg/ml proteinase K (Dako, Denmark) for 15 minutes. The following steps were performed according to the manufacturer's instructions regarding the TUNEL assay by using the ApopTag plus peroxidase in-situ apoptosis detection kit (S7110, Chemicon International, Inc., Temecula, CA, USA). Peroxidase substrate 3, 3-diaminobenzidine was used to stain for apoptotic cells. Methyl green (0.5%) was used as a nuclear stain. Four visual fields were investigated from each group using a X40 objective (Olympus, BX51, Japan). Apoptotic cells in the lateral geniculate body, optic radiatio and optic disc were counted using a graduated graticule measuring 0.25 mm^2 at this magnification. The numbers of apoptotic cells were quantified in each visual field, and the total count for the four sampled fields was expressed in mm^2.

Statistical analysis

Statistical analysis was performed using commercially available software SPSS for Windows, version 15.0, (SPSS Inc., USA). Wilcoxon signed ranks test was used for dependent variables and the Mann-Whitney U-test was used for independent variables. Data were expressed as mean ± standard deviation. A probability value less than 0.05 was accepted as statistically significant.

Results

Fig. 1 demonstrates mean ± SD values for ICP values. The difference between before and after kaolin injections regarding ICP measurements were statistically significant ($p = 0{,}027$) for both groups.

The mean value of the TNF-α measured from the blood samples of the control group and the treatment group animals were 19.2 ± 5.4 and 11.4 ± 0.7, respectively and the difference between two groups was statistically significant ($p = 0.004$). The mean value of the TNF-α measured from CSF samples of the control group and the treatment group were 18.7 ± 4.3 and 11.5 ± 3.2, respectively. The difference between two groups regarding CSF TNF- α values was statistically significant ($p = 0.016$) (Fig. 2).

The treatment group and the control group were compared for the number of terminal deoxyribonucleotide transferase-mediated dUTP nick end labeling (TUNEL) positive cells in the specimens from the optic disc, optic radiation, and the lateral geniculate body. Data revealed statistically significant

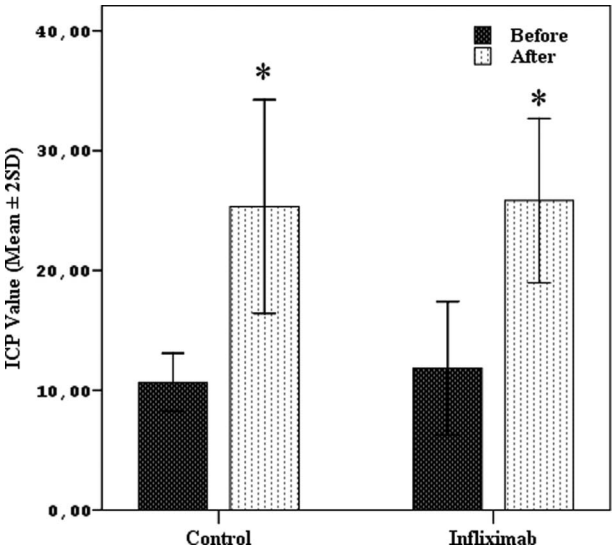

FIG. 1. Bar graph demonstrating ICP measurement differences (mean ± SD) in groups before and after kaolin injection. (*$p = 0{,}027$).

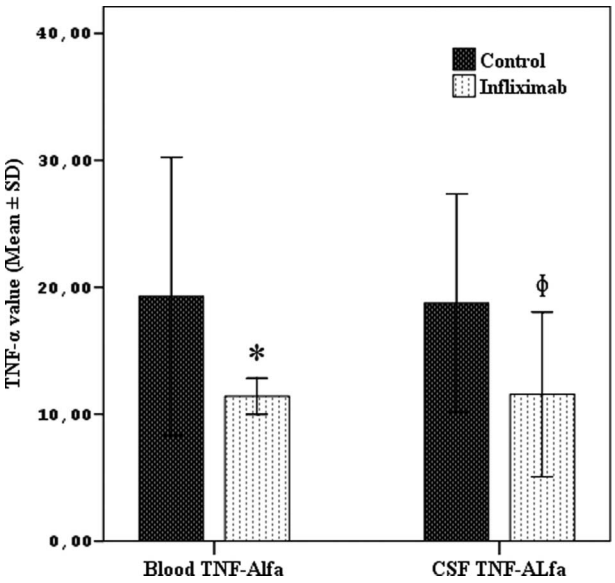

FIG. 2. Bar graph demonstrating blood and CSF TNF-α measurement differences (mean ± SD) in groups. (*$p = 0.004$; $^{\phi}p = 0.016$).

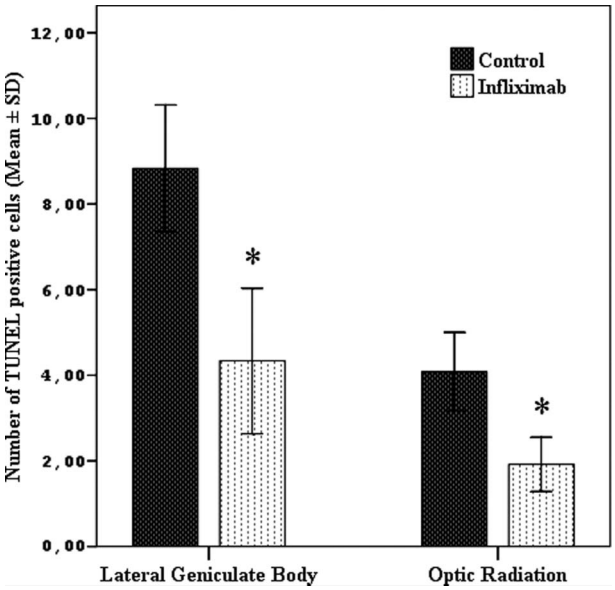

FIG. 3. Bar graph demonstrating the number of TUNEL positive cells (mean ± SD) in lateral geniculate body and optic radiation specimens. (*$p = 0.001$).

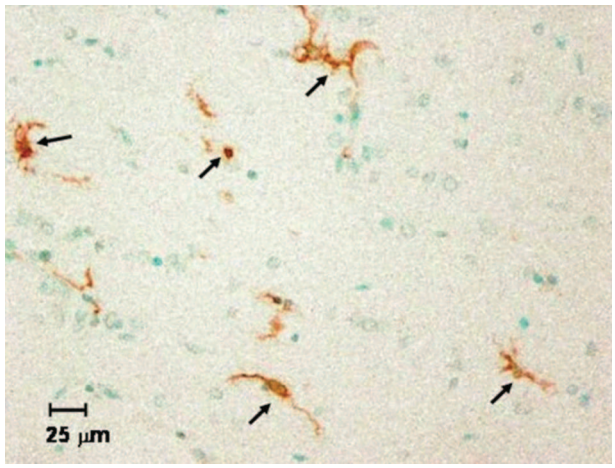

FIG. 4. Apoptotic microglial cells and scattered glial cells are shown in lateral geniculate nucleus stained brown using TUNEL method to identify cells undergoing apoptotic cell death. Arrows indicate TUNEL-positive cells. TUNEL, original magnification × 400. Scale bar = 25 μm.

difference in specimens both from the lateral geniculate body and the optic radiation regarding TUNEL positive cell counts in favor of infliximab administered group ($p = 0$, 001 for both) (Fig. 3). The number of apoptotic cells in the infliximab group was significantly less than the number of cells in the control group (Fig. 4). However, there were no apoptotic cells in the optic discs in any of the groups (Fig. 5).

Discussion

The roles of TNF-α in various types of cells have been documented,[4] whereas identification of TNF-α induced intracellular signaling in the central nervous system and under pathophysiological conditions, including hydrocephalus, remains to be a significant objective of research. The optic pathway, a region vulnerable to increased pressure and to ischemic insults, plays a pivotal role in neuronal function, as it is associated with vision function. Thus, it is worthwhile to clarify the protective effects of TNF-α monoclonal antibodies against neuronal cell death and their mechanisms in the optic pathway.

Several studies suggest that, in a significant proportion of cases, the visual pathways are among the important targets of increased ICP.[18,19] The mechanism of brain damage in hydrocephalus is known to bear some similarities to those associated with trauma and stroke.[20] In the hydrocephalus, the periventricular

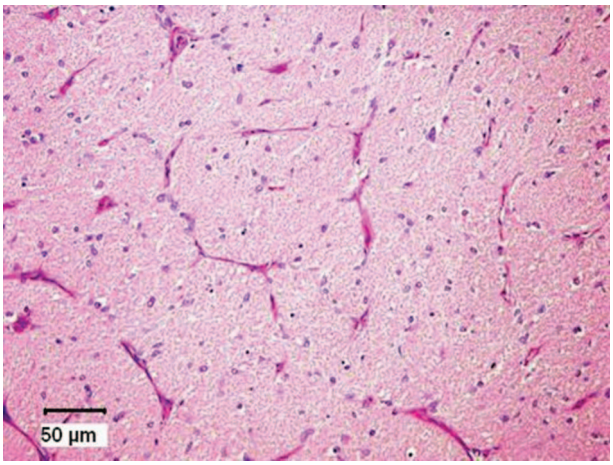

FIG. 5. Normal optic disc is shown. Haematoxylin & eosin, original magnification × 200. Scale bar = 50 μm.

tissue is characterized by disruption of the ependyma, edema, neuronal degeneration, white matter lesions, gliosis[21–23] and capillary microinfarctions.[21] It was suggested that oxidative brain damage might represent an important factor resulting from or contributing to the pathogenesis of hydrocephalus.[20,24] In the study of Socci *et al.*, MDA levels were reported to be significantly higher in the hippocampus and basal ganglia of 25-day-old hydrocephalic rats.[24]

Caner *et al.* suggested that vascular changes observed in hydrocephalic rats might be due to the high level of lipid peroxidation, which in turn might be the consequence of ischemia caused by the hydrocephalus-related stretching of cerebral vessels and reduced cerebral blood flow in the periventricular tissue.[20,25,26,27] Mori *et al.* indicated that the production of superoxide dismutase, one of the scavengers of oxygen species, was reduced congenitally hydrocephalic rat brain.[28] Miller *et al.* reported that reactive astrocytosis and microgliosis are associated with progressive untreated ventriculomegaly, but that shunt treatment can reduce the gliosis occurring with hydrocephalus.[29]

In the present study, the high intrathecal and blood levels of TNF- α was observed in hydrocephalic rabbits. These results are compatible with the previous reports.[30–32] The reason of this increased TNF- α level could be due to damage in blood brain barrier, to an increased production of this cytokine, or to its decreased elimination.[4] Previously, the increased production of TNF- α has been shown in the pathophysiology of SCI.[21] Harrington *et al.* demonstrated that 6 h postacute SCI, there was an increased neuronal expression of TNF-α and its receptors. They also demonstrated that TNF-α level in the cerebrospinal fluid were significantly increased after acute SCI.[33]

The benefits of several monoclonal antibodies against TNF-α have been established in various animal models.[34,35] Infliximab is a chimeric monoclonal antibody that blocks the effects of TNF- α. Infliximab also inhibits the production of other proinflammatory cytokines, reducing cell infiltration, having then a potent anti-inflammatory agent. Onset of the clinical effect is usually seen within days.[36,37] The mechanism of action may include neutralization of TNF, lysis of activated immune cells,[38] and induction of apoptosis in activated macrophages[39] and T cells.[40] Previously, it has been revealed that infliximab has protected the spinal cord from the compression injury.[41] In the present study, we have demonstrated that usage of infliximab resulted in a significant reduction in neurological injury of optic pathways except optic disc on a kaolin induced hydrocephalus model.

Yinon *et al.* have previously shown that the optic tract, chiasm, nerve and retina had been morphologically and histologically normal in a hydrocephalus cat model.[42] Likewise, we could not demonstrate apoptosis in optic nerve. The TUNEL (+) staining was significantly less on optic radiation and the lateral geniculate body, indicating that there was increased neural protection. These findings are compatible with previous reports that TNF- α blockage results in a superior neurological outcome.[31,41,43] Although our study does not elucidate the mechanisms, it does affirm that TNF- α blockage exerts a neuroprotective effect. As a future evolution, analysis of infliximab treatment at different time point's with functional outcomes and multiple sites in the CNS including areas of the cerebral cortex would be valuable to inform clinical use.

Conclusion

In summary, it appears that kaolin induced hydrocephalus is resulting in increased levels of TNF- α in the CSF, and cellular death signaling on the optic radiation and the lateral geniculate body. This study suggests that infliximab may have a neuroprotective effect through its anti-apoptotic property on hydrocephalus induced optic pathway injury. However, because this study reflected effects of infliximab at 2 weeks after hydrocephalus formed, we could not define the time of maximal effect and duration of action. Further studies are needed to evaluate the effects of infliximab on hydrocephalus induced optic pathway injury.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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