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Tacrolimus depresses local immune cell infiltration but fails to reduce cortical contusion volume in brain-injured rats

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Abstract

The immunosuppressant drug tacrolimus (FK-506) failed to show an anti-edematous effect despite suppressing pro-inflammatory cytokines in cerebrospinal fluid following focal traumatic brain injury. By questioning the role of the inflammatory response as a pharmacological target, we investigated the effects of FK-506 on immune cell infiltration in brain-injured rats.

Following induction of a cortical contusion, male Sprague–Dawley rats received FK-506 or physiological saline intraperitoneally. Brains were removed at 24 h, 72 h or 7 days, respectively. Frozen brain sections (7 μm) were stained immunohistologically for markers of endothelial activation (intercellular adhesion molecule-1 – ICAM-1), neutrophil infiltration (His-48), and microglial and macrophage activation (Ox-6; ED-1), respectively. Immunopositive cells were counted microscopically. Contusion volume (CV) was quantified morphometrically 7 days after trauma.

Inflammatory response was confined to the ipsilateral cortex and hippocampal formation, predominating in the contusion and pericontusional cortex. Strongest ICAM-1 expression coincided with sustained granulocyte accumulation at 72 h which was suppressed by FK-506. Ox-6+ cells prevailing at 72 h were also significantly reduced by FK-506. ED-1+ cells reaching highest intensity at 7 days were significantly attenuated at 72 h. Cortical CV was not influenced.

FK-506 significantly decreased post-traumatic local inflammation which, however, was not associated with a reduction in cortical CV. These results question the importance of post-traumatic local immune cell infiltration in the secondary growth of a cortical contusion.

Keywords: Traumatic brain injury; ICAM-1; Microglia; Macrophages; Granulocytes

Abbreviations: APC, antigen presenting cells; b, back; c, contusion; cA, area of contusion of slice A; CCI, controlled cortical impact; clc, contralateral cortex; clh, contralateral hippocampus; CV, contusion volume; f, frontal; ICAM, intercellular adhesion molecule; IL, interleukin; ilh, ipsilateral hippocampus; pc, pericontusional area; ROI, region of interest; TBI, traumatic brain injury; TNF, tumor necrosis factor; TTC, tri-phenyl-tetrazolium-chloride

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Introduction

Traumatic brain injury (TBI) induces a plethora of pathophysiologically important alterations contributing to progressive structural and functional impairment. Among other factors, sustained local inflammation (Feuerstein et al., 1997; Raivich et al., 1999) contributes to secondary injury by reducing perfusion due to leukocyte adherence and thrombocyte aggregation (Price et al., 2003) and by liberating autodestructive mediators known to activate second- and third-messenger cascades which, in turn, up-regulate expression and release of chemokines, adhesion molecules, and pro-inflammatory cytokines (Allan and Rothwell, 2001; Price et al., 2003). Within the first few days after injury, these events guide circulating neutrophilic granulocytes, monocytes, and macrophages to the site of damage and stimulate resident immunocompetent cells to remove debris. Over time, adapted changes mediate regional confinement of underlying damage, thereby possibly contributing to reorganization (Price et al., 2003; Vela et al., 2002). Following TBI, the inflammatory response begins within hours after injury and lasts up to several days and even weeks. There is still controversy in how far immune response and the recruitment of immune cells contributes to secondary injury growth or promotes regenerative processes (Lenzlinger et al., 2001; Morganti-Kossmann et al., 2001; Shohami et al., 1999).

The immunosuppressant lipophilic drug tacrolimus (FK-506) attenuates the early inflammatory response in a variety of different organs (e.g. retina, liver, intestines, brain) by decreasing activated T-lymphocytes (Fee et al., 2003; Masri, 2003), attenuating leukocyte accumulation (Tsujikawa et al., 1998), neutrophil infiltration (Garcia-Criado et al., 1997), and reducing activation of resident immunocompetent cells, e.g. hepatic natural killer cells (Tamura et al., 1998) and microglia (Kaminska et al., 2004). Moreover, FK-506 inhibits release of pro-inflammatory cytokines (Masri, 2003; Stover et al., 2001) and attenuates expression of endothelial inter-cellular adhesion molecule-1 (ICAM-1) (Squadrito et al., 1999).

Contrary to experimental stroke models in which tacrolimus significantly reduced infarct volume and edema formation, and improved neurological outcome (Benetoli et al., 2004; Bochelen et al., 1999), tacrolimus failed to exert similar effects using a comparable study protocol following experimental TBI (Scheff and Sullivan, 1999; Stover et al., 2001). Despite a complete suppression of cerebrospinal fluid levels of the pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) (Stover et al., 2001) suggest tacrolimus-induced immunosuppression following TBI. It still remains to be assessed if tacrolimus reduces the post-traumatic local cellular response as seen following cerebral ischemia (Squadrito et al., 1999; Tamura et al., 1998; Tsujikawa et al., 1998) and liver ischemia (Garcia-Criado et al., 1997). Since activation of immunocompetent cells contributes to evolving damage and inhibition of these cells attenuates post-ischemic structural and functional injuries, the absent protective, i.e. tissue-sparing, effect following experimental TBI could be related to a failure of tacrolimus in attenuating the local immune cell infiltration. This hypothesis was tested by investigating the changes in ICAM-1, neutrophilic granulocytes, microglia, and macrophages at 24 h, 72 h, or 7 days following controlled cortical impact (CCI) injury in tacrolimus-treated rats compared to control rats receiving physiological saline.

Materials and methods

Study protocol

Following approval of the experimental protocol by the committee for animal research in Berlin, Germany, 46 brain-injured male Sprague–Dawley rats (250–350 g) were randomly assigned to receive tacrolimus or physiological saline. At 24 h, 72 h, or 7 days after injury with 5 rats per time point and treatment group (total of 30 animals), brains were removed for immunohistochemical analysis. In another group with a total of 16 animals, contusion volume (CV) was quantified using tri-phenyl-tetrazolium-chloride (TTC) staining following placebo ($n = 8$) and tacrolimus injection ($n = 8$), respectively.

Surgical procedure and CCI injury

A total of 46 spontaneously breathing rats anesthetized with a mixture of isoflurane, N_2O, and O_2 (1.5–2.5 vol.% isoflurane, 0.5 l/min N_2O, 0.3 l/min O_2) were subjected to a focal contusion to the left parieto-temporal cortex with the CCI injury device as previously described (Stover et al., 2001; Thomale et al., 2006). In detail, anesthetized rats were positioned prone on a feedback regulated heating pad, maintaining body temperature at 37 °C and the head was secured in a stereotactic frame. A midline incision of about 2 cm was performed reaching from the level right behind the eyes to the craniocervical junction. After exposing the left side of the skull, the temporal muscle was dissected and removed. Under microscopic view and continuous irrigation and suction with physiological saline, a left craniectomy was performed using the anatomical landmarks of the coronal, sagittal, and lambdoid sutures and on the temporal bone down to the posterior portion of the zygomatic arch. The size of the trephination was $8 \times 8\,mm^2$. The dura remained intact in all animals following this procedure. The tip of the pneumatic-driven bolt (5 mm in diameter) of the CCI device was