MRI Determination of the Mechanisms Underlying TNF-α-induced Changes in Cerebral Blood Volume, Tissue Water Diffusion and BBB Permeability.

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Many neuropathologies are associated with elevated cerebral TNF-α, but the consequences of such high expression are unresolved. Using MRI we demonstrate that a focal intrastriatal injection of TNF-α causes a significant, acute reduction in cerebral blood volume that is dependent on TNF-α receptor type 2 (TNFR2) activation, and can be ameliorated by pre-treatment with an endothelin-receptor antagonist. In contrast, delayed blood-brain barrier breakdown and reduction in tissue water diffusion appear to be mediated by both TNF-α receptor pathways. These results identify endothelin receptors and TNFR2 as potential therapeutic targets in TNF-α-associated neuropathologies. Thus, MRI has contributed significantly to our understanding of TNF-α effects within the CNS.

Introduction

Elevated cerebral TNF-α is associated with a wide range of neuropathologies, but the consequences of this remain unclear. We have previously demonstrated that an intrastriatal injection of TNF-α causes an acute reduction in regional cerebral blood volume (rCBV) followed by a delayed reduction in the apparent diffusion coefficient (ADC) of tissue water and breakdown of the blood-brain barrier (BBB)[1]. The aim of this study was to determine the pathways and mediators induced by TNF-α that are responsible for the observed changes in rCBV, ADC and BBB.

Peripheral injection of TNF-α into rats significantly increases plasma endothelin (ET) levels[2]. Therefore, in this study we used a non-specific ET receptor antagonist[3] to determine the effect on the acute rCBV reduction, and the delayed changes in ADC and BBB.

TNF-α binds to two receptors, TNFR1 (p55) and TNFR2 (p75)[4], which are thought to activate distinct signaling pathways. While rat TNF-α (rrTNF-α) binds non-specifically to both TNF-α receptors, human TNF-α (rhuTNF-α) will bind only to TNFR1 in rat brain[5,6]. Thus, in this study we used rhuTNF-α to selectively activate TNFR1 to determine which pathway is responsible for the observed effects on rCBV, ADC and BBB.

Methods

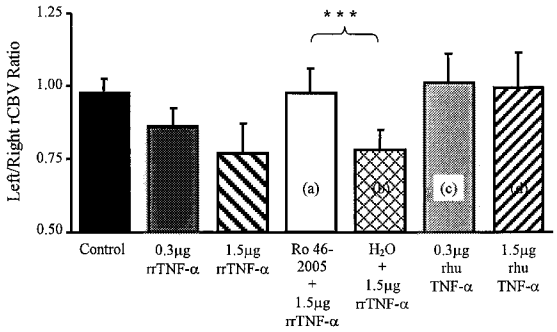
Male Wistar rats (218±20g) were anaesthetised with hypnorm/hypnovel and underwent one of the following treatments: (a) i.v. injection of the ET receptor antagonist Ro 46-2005 (1mg/0.25ml sterile water) 10min before intracerebral injection of 1.5μg/1μl rrTNF-α (n=10); (b) i.v. injection of sterile water (0.25ml) 10min before intracerebral injection of 1.5μg/1μl rrTNF-α (n=4); (c) intracerebral injection of 0.3μg/1μl rhuTNF-α (n=5); and (d) intracerebral injection of 1.5μg/1μl rhuTNF-α (n=5). Six animals from group (a) and all from group (b) were imaged 1.5h after intracerebral injection and then sacrificed. The additional 4 animals from group (a) were imaged at 24h. All animals from groups (c) and (d) were imaged at both 1.5h and 24h after intracerebral injection.

MRI was performed in a 7T magnet with a Varian Inova spectrometer. Animals were positioned in an Alderman-Grant resonator using a custom-built bite-bar, and anaesthesia was maintained throughout with 1.0-1.5% halothane in 70%N_2O/30%O_2. At 1.5h and 24h a time series of GE images (1mm slice, TR=20ms, TE=10ms, 1.2s per image) were acquired during which 100μl of a gadolinium-based contrast agent (Gd) was injected into a tail vein. Time course signal changes were converted into a map of rCBV by tracer kinetic analysis. T_1-weighted images (TR=500ms, TE=20ms) were acquired 10min post-Gd to determine BBB permeability. At 24h diffusion-weighted images were also acquired (2mm slice, TR=1s, TE=41ms, δ/Δ=12/22ms, b=125, 750, 1500smm^{-2}). Diffusion gradients were applied separately along three orthogonal axes and ADC "trace" maps were calculated. These data are compared with data acquired previously[1] from animals injected with either vehicle solution (0.1% BSA/saline), 0.3μg rrTNF-α, or 1.5μg rrTNF-α.

Results

The reduction in striatal rCBV observed 1.5h after intracerebral injection of rrTNF-α was eliminated in animals pre-treated with Ro 46-2005 but not sterile water (see Figure). The difference between injected/non-injected striatal rCBV ratios for the two groups was highly significant (unpaired t test, P<0.005). Animals injected with either 0.3μg or 1.5μg rhuTNF-α exhibited no reduction in rCBV at 1.5h (see Figure).

Figure: rCBV ratios obtained 1.5h after TNF-α injection.



The reduction in ADC found in animals injected with rrTNF-α was not affected by pre-treatment with Ro 46-2005 (see Table). Similarly, a significant difference was found between the striatal ADC values in animals injected with 1.5μg rhuTNF-α (see Table). However, although a reduction in ADC was apparent in 3/5 animals injected with 0.3μg rhuTNF-α, this did not reach significance (paired t test, P=0.136).

Table: ADCs obtained at 24h after intracerebral injection of TNF-α.

Vehicle		0.3μg rrTNF-α		1.5μg rrTNF-α		Ro46-2005 + 1.5μg rrTNF-α		0.3μg rhuTNF-α		1.5μg rhuTNF-α	
L	R	L	R	L	R	L	R	L	R	L	R
6.78 ± 0.35	6.79 ± 0.35	6.28** ± 0.38	6.99 ± 0.46	6.30** ± 0.52	6.90 ± 0.35	6.72* ± 0.25	7.23 ± 0.17	7.15 ± 0.28	7.33 ± 0.26	6.76** ± 0.20	7.14 ± 0.31

All values are Mean ● S.D. x10^{-4}mm^2/sec. *P<0.05, **P<0.02. L=left, R=right.

Pre-treatment with Ro 46-2005 did not significantly affect the level of BBB breakdown observed in animals injected with 1.5μg rrTNF-α (post-Gd T_1 signal intensity increase = 10.3±2.7% vs. 8.1±2.1% in untreated group). However, in animals injected with 1.5μg rhuTNF-α the degree of BBB breakdown was substantially reduced (signal intensity increase = 5.2±1.6%), which may reflect the reduced monocyte recruitment (95±33/mm^2) compared to the rrTNF-α group (189±7/mm^2).

Discussion

In this study we have shown that a focal, intrastriatal injection of TNF-α in the rat brain results in an acute, dose-dependent reduction in cerebral blood volume that is mediated by endothelin, and requires activation of the TNF-α receptor 2 pathway. These data suggest that both endothelin receptors, and the TNFR2 pathway, are potential targets for therapeutic intervention in TNF-α-associated neuropathologies. We found no evidence that either the delayed reduction in tissue ADC or BBB breakdown is mediated by endothelin. However, the ET system is widespread in the brain, with ET and its receptors being expressed by vascular, neuronal and glial cells. Since the Ro 46-2005 was administered intravenously it may not antagonise deep parenchymal effects of ET. The ADC and BBB data acquired from animals injected with rhuTNF-α suggest that both receptor pathways contribute to the processes underlying the delayed reduction in tissue ADC and BBB breakdown. MRI is the only methodology that enables measurement of all of these physiological parameters *in vivo*, and, thus, has contributed significantly to our understanding of TNF-α effects within the CNS.

References

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