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Research Report

Neuronal tumour necrosis factor-α and interleukin-1β expression in a porcine model of intracerebral haemorrhage: Modulation by U-74389G

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ARTICLE INFO

Article history:
Accepted 17 April 2015
Available online 25 April 2015

Keywords:
Intracerebral haemorrhage
Porcine model
TNF-α
IL-1β
U-74389G

ABSTRACT

Tumour necrosis factor α (TNF-α) and interleukin 1β (IL-1β) are important mediators of intracerebral haemorrhage (ICH) inflammatory response. Lazaroids, established antioxidants and neuroprotectants, have been studied in several brain pathologies. The present study was designed to investigate: a) TNF-α and IL-1β changes, in neurons and b) U-74389G effects, 4 and 24 h after haematoma induction in a porcine model of intracerebral haemorrhage.

In twenty male landrace pigs (swines) aged 135–150 days old, autologous whole blood was injected around the right basal ganglia territory; in ten of the pigs the lazaroid compound U-74389G was administered. Brain TNF-α and IL-1β immunopositive neurons were determined by immunoarray techniques at 4 and 24 h timepoints.

After the haematoma induction the number of TNF-α immunopositive neurons ipsilateral to the haematoma was significantly higher compared to the contralateral site at 4 h ($p<0.0005$), while U-74389G significantly reduced the number of TNF-α immunopositive neurons, ipsilateral to the haematoma, at 4 h ($p=0.002$); at 24 h, TNF-α immunopositive neurons were found significantly lower in the control group ipsilateral to the haematoma in comparison to 4 h timepoint ($p<0.0005$).

The number of IL-1β immunopositive neurons at 4 h after the hematoma induction was significantly higher ipsilateral to the haematoma site ($p<0.0005$). U-74389G had no statistical significant effect.

TNF-α and IL-1β, increase in neurons, 4 h after the haematoma induction, ipsilateral to the haematoma site. The administration of the antioxidant compound U-74389G, results in early

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http://dx.doi.org/10.1016/j.brainres.2015.04.034

(at 4 h) decrease of TNF-α immunopositive neurons but shows no statistical significant effect to IL-1β immunopossitive neurons.

1. Introduction

Intracerebral haemorrhage represents a clinical entity with high incidence, mortality and morbidity. It is estimated that it represents 10–15% of all cases of stroke, and has higher mortality and morbidity rates in comparison to ischaemic stroke, whilst on the other hand, recent data suggests that the incidence of ICH is approximately twice the incidence of subarachnoid haemorrhage (Broderick et al., 1993). The mortality rate of ICH is high, estimated at approximately 50% (Broderick et al., 1994; Rodríguez-Yáñez et al., 2013). According to the World Health Organization, approximately 15.3 million strokes occur every year worldwide, amongst them, 2–3 millions are haemorrhagic. Despite the high incidence, ICH still remains a cerebrovascular disease, not fully understood in terms of pathophysiology (Van Asch et al., 2010; Qureshi et al., 2009; Cannon et al., 2007). ICH is a pathophysiologically different entity of traumatic brain injury, ischaemic brain injury and neurodegenerative diseases. Following the occurrence of ICH, rapid accumulation of blood within the brain parenchyma leads to a disruption of the local central nervous system (CNS) anatomy and increase in local pressure (primary injury) within minutes to hours from the onset of bleeding (Qureshi et al., 2009). It is the second phase (secondary injury) that majorly accounts for the complexity of the ICH pathophysiology, as the entrapped intraparenchymal blood cytotoxicity along with the activated hypermetabolic, pro-inflammatory and excitotoxic pathways leads to oxidative stress, inflammation, irreversible disruption of the blood–brain barrier, activation of the surrounding microglia, cellular oedema and neuronal apoptosis (Aronowski and Zhao, 2011; Qureshi et al., 2009).

TNF-α and Interleukin IL-1β are two of the most important proinflammatory cytokines involved in the inflammation process (Gong et al., 2000), apoptosis (Matsushita et al., 2000) and blood brain barrier disruption (Xi et al., 2001a, 2001b; Wagner et al., 1999), playing an important role in ICH pathophysiology (Holmin and Mathiesen, 2000; Hua et al., 1998). TNF-α in traumatic brain injury and ischaemic stroke seems to play important role by a number of different mechanisms (Lambertsen et al., 2012; Lu et al., 2009; Lenzlinger et al., 2001).

Both the above cytokines seem to exacerbate neuronal loss in ICH (Katsuki, 2010; Wang and Doré, 2007; Karwacki et al., 2006) with TNF-α being secreted by neurons, activated microglia and astrocytes, leading to acute phase protein production, vasogenic oedema formation and matrix metalloproteinases formation and IL-1β, being produced and secreted by microglia and astrocytes, resulting in vasogenic oedema formation and MMPs secretion. At present there is no clear recognition of the role of these cytokines in ICH pathways of secondary injury especially in microglia, neurons and macrophages (Zarros et al., 2014).

In the treatment of ICH several antioxidants have been used and recently reviewed (Villa and Gorini, 1997) but so far not the antioxidant family of lazaroids. Lazaroids are 21-aminosteroids compounds with similar to corticoids actions but without their side effects (Hall, 1988a). Current data suggests that these compounds among others act as free radical scavengers (Hall et al., 1991, 1994; Hinzmann et al., 1992; Sato and Hall, 1992; Hall, 1988b), stabilize the lipid bilayer of the membrane, resulting in defence against lipid peroxidation (Hall et al., 1994; Hinzmann et al., 1992), attenuate glutamate toxicity, TNF-α, cytokines, in CNS pathologies (Kabadere et al., 2004; Altavilla et al., 1998) and suspend glioma's cells multiplication. Their action is believed to be cytotoxic rather than cytostatic (Durmaz et al., 1999).

The lazaroid U-74389G compound is an inhibitor of lipid peroxidation in a variety of systems, including cultured brain microvessel endothelial cells, Cu^{2+} treated monocytic THP1 (Acute monocyte leukaemia cell line) cells, and LLC-PK1 cell layers (porcine kidneys epithelial cell line). This compound has also been shown to prevent organ damage in intestinal cold storage preservation and reperfusion injury (Campo et al., 1996; Salahudeen et al., 1995; Katz et al., 1995).

From experiments in rats it has been shown that U-74389G reduces the infarct in permanent middle cerebral artery occlusion models (Acka et al., 2007), is neuroprotective in embryonic ventral mesencephalic tegmental dopaminergic neurons in cold storage (Thajeb et al., 2006), and reduces the paraquat-induced apoptosis into the hippocampus (Melchiorri et al., 1998).

Similarly, it acts neuroprotectively in experimental subarachnoid haemorrhage (Turner et al., 1999), protects dopamine neurons from death due to oxidative stress in vitro (Stull et al., 2002), and prevents beta-(25–35) toxicity in cortical neurons (Lucca et al., 1997) and transient global ischaemic damage in hippocampus (Tseng et al., 1997).

Other studies suggest that U-74389G protects normal brain from stereotactically induced radiation (Buatti et al., 1996) and prevents arachidonate-induced lipid peroxidation and permeability alterations in brain microvessel endothelial cells (Shi et al., 1995).

The aim of the present study was to investigate a) the early (4 and 24 h) expression of two proinflammatory cytokines, TNF-α and IL-1β in the neurons at the perihaematomal and the identical contralateral area after the induction of ICH in a large porcine model (that better simulates the brain pathophysiology of ICH) and b) the possible modulatory effects of the antioxidant lazaroid U-74389G 4 and 24 h after the experimental haematoma induction.

2. Results

Haematoma was macroscopically recognized deep seated around the basal ganglia territory of the right hemisphere.