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Fenofibrate, a peroxisome proliferator-activated receptor α agonist, exerts neuroprotective effects in traumatic brain injury

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Abstract

Peroxisome proliferator-activated receptor α (PPARα) has been demonstrated to reduce inflammation in various inflammatory diseases. As traumatic brain injury (TBI) caused a neuroinflammatory response, we examined the effect of fenofibrate, a PPARα agonist, on the post-traumatic consequences caused by lateral fluid percussion of brain in rats. The effects of fenofibrate (50 and 100 mg/kg) were evaluated on the consequences of TBI in the early phase (6 and 24 h) and the late phase (7 days) after TBI. Neurological deficit, brain lesion, cerebral oedema and ICAM-1 expression were evaluated. Treatment with fenofibrate (given p.o. at 1 and 6 h after TBI) decreases the neurological deficit induced by TBI at 24 h. Furthermore, fenofibrate reduces brain oedema and ICAM-1 expression at 24 h post-TBI. Rats given fenofibrate at 1, 6, 24, 48 and 72 h after TBI show neurological recovery associated with a reduction of the brain lesion at 7 days post-TBI. The present data represents the first demonstration that fenofibrate, a PPARα agonist, exerts neuroprotective effects in TBI. The activation of receptor PPARα could be beneficial by counteracting the deleterious inflammatory response following TBI. This suggests that PPARα activation could be a new and promising therapeutic strategy for the treatment of brain trauma.

Keywords: Cerebral oedema; Fenofibrate; Inflammation; Neuroprotection; Peroxisome proliferator-activated receptor α; Traumatic brain injury

Traumatic brain injury (TBI) remains one of the leading causes of death and disability in industrialized countries [3]. Despite numerous studies on animal models of TBI searching for therapeutic strategies, no neuroprotective therapy is currently available.

Peroxisome proliferator-activated receptor α (PPARα) is one of the three subtypes of the nuclear receptor PPAR family [15]. PPARs are implicated in several physiological processes, such as the regulation of lipoprotein, lipid metabolism and glucose homeostasis. Recent observations indicate that PPARα activators could reduce the inflammation induced in different inflammatory pathologies including asthma [31], hypertensive heart disease [9,20], hepatic inflammation [12] and cerebral ischemia [7]. As TBI induces also a deleterious neuroinflammatory response, it might be hypothesized that PPARα activation would be beneficial by counteracting this post-traumatic neuroinflammation. In order to investigate the role of PPARα activation on the consequences of brain trauma, we studied the effect of fenofibrate, a known PPARα activator, on the neurological deficit in the early (6 and 24 h) and the late phase (7 days) after TBI, on the cerebral oedema and the brain lesion caused by TBI. Additionally, we examined its effect on the expression of the inducible adhesion molecule, InterCellular Adhesion Molecule-1 (ICAM-1), used as a marker of post-traumatic inflammatory event.

Animals and materials: Animal care complied with the French regulations covering the protection of animals used for experimental and other scientific purposes (D2001-486), with the European Community regulations (Official Journal of European Community L358 12/18/1986),

Abbreviations: BWC, brain water content; COX2, cycloxygenase type 2; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; NOS2, nitric oxide synthase type 2; PBS, phosphate-buffered saline; PPARα, peroxisome proliferator-activated receptor α; TBI, traumatic brain injury; TNFα, tumour necrosis factor alpha

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and it conformed to the *Guide for the Care and Use of Laboratory Animals* published by U.S. National Institutes of Health (NIH Publication no. 85–23, revised 1996). Male Sprague–Dawley rats were supplied by Iffa-Credo (L'Arbresle, France). Fenofibrate, methylcellulose, hydrogen peroxide, normal goat serum, diaminobenzidine, gelatin and sucrose were purchased from Sigma Chemicals Corporation (Saint Quentin-Fallavier, France). Mouse antibody raised against rat CD54, clone 1A29 (MCA773) was from Serotec (Cergy St Christophe, France) and biotinylated goat anti-mouse rat adsorbed antibody (AP-181B) from Chemicon (Euromedex, Mundolsheim, France). Streptavidin–biotin peroxidase complex (Vectastain Elite ABC) was obtained from Dako (Trappes, France).

Fluid percussion-induced brain injury: Male Sprague–Dawley rats (weighing 300–350 g) were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and placed on a stereotaxic frame. During surgery, animals were positioned on a heating blanket (Harvard, UK) to maintain body normothermia ($37.5 \pm 0.5\,^{\circ}C$). Traumatic brain injury of moderate severity was induced by fluid percussion using the protocol previously described [2]. The scalp was incised and a 3 mm craniotomy was made lateral to the right temporoparietal cortex (coordinates: 3.5 mm anterior and 6 mm above the interaural line [21]) with a dental drill taking care to leave the dura mater intact. A 3 mm diameter polyethylene tube was placed over the dura mater, fixed securely into the craniotomy site with dental cement (Perfex, USA) and connected to a solenoid valve (Danfoss, Denmark). The opposite end of the valve was connected to a high performance liquid chromatography pump (Gilson). The system was filled with sterile water, providing a calibrated outflow pressure of 1.6–1.8 bar. A solenoid valve opening for 20 ms and controlled with a timer (Omron, Japan), triggered the percussion directly onto the dura mater. The applied cortical pressure was measured extracranially by a pressure transducer (Emka Technologies, France) connected to an oscilloscope (DSO 400, Gould, France). Immediately after fluid percussion, the tube was removed, the scalp sutured, and the animal was returned to its home cage warmed at 26–28 °C to recover from the anaesthesia. Thereafter, rats were group-housed under temperature- and light-controlled conditions with food and water ad libitum. Sham-operated rats underwent the same surgery except for percussion.

Neurological function: A neurological examination was performed in a blinded fashion using a grading scale (Table 1) [28]. Contralateral sensorimotor functions were examined by assessing placing reactions (leg hanging and visual), grasping reflex, righting reflex ("head tilted") in rats placed on a table. Rats were also examined for abnormal postures (forelimb flexion and thorax twisting). The scores for each item were summed and used as a global neurological score; the maximum was 9 for non-operated rats.

Brain lesion: Rats were anaesthetised with pentobarbital and killed by decapitation. Their brains were promptly removed, frozen in isopentane and stored at −40 °C. Serial coronal sections (50 μm thick) were cut in a cryostat (−15 °C) at 1 mm intervals, beginning at the level 13.7 to 1.7 mm relative to the interaural line [21]. The sections were stained with cresyl violet. The lesion areas were measured with an image analyzer (IMSTAR, France).

Table 1
Neurological examination grading scale

Item		Normal score	Deficit
Placing reactions			
Leg hanging	Left forepaw	1	0
	Left hindpaw	1	0
Visual		1	0
Grasping reflex	Left forepaw	1	0
	Left hindpaw	1	0
Righting reflex			
Head tilted	Left side	1	0
	Right side	1	0
Abnormal postures		Absent	Present
Thorax twisting		1	0
Left forelimb flexion		1	0
Global neurological score		9	

Brain oedema: Cerebral oedema was determined by measuring brain water content (BWC) using the wet weight–dry weight technique [1], and the results are expressed as a percentage of the water content. Rats were killed by an overdose of sodium pentobarbital (200 mg/kg, i.p.). The brains were promptly removed and a thick (4 mm) coronal slice was taken at the temporoparietal level. The slice was removed from the ipsilateral hemisphere. The fresh tissue samples were immediately weighed (wet weight) and placed in an incubator at 100 °C for 24 h. The samples were weighed once again to determine the dry weight. The BWC was calculated as follows:

$$\mathrm{BWC} = \left(\frac{(\text{wet weight} - \text{dry weight})}{\text{wet weight}} \right) \times 100$$

Preparation of brain tissue for immunohistochemistry: Rats were anaesthetised with sodium pentobarbital and perfused transcardially with 200 ml of heparinized saline followed by 400 ml of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) containing 4% paraformaldehyde. The brains were then removed, kept for 1 h in the same fixative solution, and placed in two successive 10% sucrose solutions, each for 24 h. The brains were rapidly frozen in isopentane and stored at −40 °C until used. Serial coronal sections were cut (20 μm) on a cryostat (−18 °C, Jung CM 3000, Leica), collected on gelatine-coated slides, and processed for immunohistochemistry.

Immunohistochemistry of ICAM-1: All sections were incubated for 10 min in 0.3% hydrogen peroxide–10% ethanol in PBS before the primary antibody was added in order to quench the endogenous peroxidase activity. Non-specific binding sites were blocked using 2% normal goat serum in PBS for 30 min. Sections were then incubated overnight at 4 °C with the primary antibody (Mouse antibody against