In vivo imaging of brain endothelium activation with a novel targeted iron-oxide based contrast agent - LacNAc-CLIO

S. van Kasteren[1], D. Anthony[2], S. Campbell[2], D. Morsali[2], B. Davis[1], N. Sibson[3]

[1]Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom, [2]Pharmacology, University of Oxford, Oxford, United Kingdom, [3]University Laboratory of Physiology, University of Oxford, Oxford, United Kingdom

Introduction

Inflammation is the body's reaction to infection or injury. One of the earliest events in an inflammatory response is the localized expression of specific adhesion molecules on endothelial cells which enables the migration of leukocytes from the bloodstream into the surrounding tissue. In the case of the brain, these adhesion molecules facilitate crossing of the blood-brain barrier. In cytokine-induced inflammation the primary adhesion molecules that are induced on endothelial cells are E- and P- selectin and their counter-receptors are expressed on a range of inflammatory cells - neutrophils, monocytes, eosinophils, and lymphocyte subsets. Each selectin receptor molecule contains a lectin-like N-terminal domain that is directly involved in mediating cell-cell contact through interactions with cell-surface carbohydrates, such as Sialyl Lewis^X (sLe^X). We have previously demonstrated that a novel gadolinium-based contrast agent (Gd-DTPA-B(sLe^X)A)[1], which contains an sLe^X mimetic moiety enabling binding to E-selectin, can be used to identify early endothelial activation in a cytokine-induced model of CNS inflammation[2]. We are now developing new agents based on an amine-functionalised cross-linked iron oxide (CLIO) particle, and here demonstrate the *in vivo* application of *N*-acetyllactosamine-CLIO-particles (LacNAc-CLIO), which are a truncated variant of an sLe^X -derivatised CLIO-particle.

Methods

LacNAc-CLIO Synthesis: The synthesis of the LacNAc-CLIO employs the 2-methoxy-2-imido-linker (IME) system[3], which has previously only been used with monosaccharides. We have now extended this system for use of the linker-precursor in enzymatic extension-reactions and for chemically synthesised polysaccharides[4]. The CLIO particles used are in the 100-500nm range, and the LacNAc-CLIO particles were also FITC-labelled to enable immunocytochemical visualisation.

Animal Preparation: Male Wistar rats (200-250g; n=2) were anaesthetised with 2.5% isoflurane in 70%$N_2$0/30%O_2 and placed in a stereotaxic frame. Each animal was microinjected with 1μg of rat recombinant interleukin-1β(IL-1β) in 1μl 0.1% BSA/saline in the left striatum (see fig. 1a for injection site), using a 50μm-tipped glass pipette. At 3.5h after IL-1βinjection, animals were positioned in a quadrature birdcage resonator with an in-built stereotaxic frame and a cannula positioned in one tail vein. The animals were then placed in the magnet and anaesthesia was maintained throughout with 1.7% isoflurane in 70%N_2O/30%O_2.

MRI: MRI was performed in a 7T horizontal-bore magnet with a Varian Inova spectrometer. The location of the injection site was determined from scout images, and a target coronal slice positioned through this site. At 4h after IL-1βinjection, 300μl of the novel contrast agent was injected via the cannulated tail vein over 2min. Fast spin-echo T_2-weighted images (1mm slice, TR=3sec, TE=37.5ms, Av=1, matrix=128x125, FOV=4cmx4cm) were acquired prior to contrast agent injection and at t=0, 10, 20 and 30min after injection. In a second group of animals (n=3) the conventional contrast agent, gadodiamide (gd), was injected and post-gd T1-weighted (1mm slice, TR=500ms, TE=20ms, Av=8, matrix=128x128, FOV=3cmx3cm) images acquired to verify patency of the blood-brain barrier (BBB).

Immunocytochemistry: Anti-FITC positive vessels were identified on 10μm thick sections counterstained with cresyl violet.

Results

Figure 1a shows T_2-weighted images acquired from an animal injected with the LacNAc-CLIO agent at t=0 and t=30min after administration of the contrast agent. Over the 30 min period, a number of dark blood vessels (fig. 1b) gradually become apparent in the injected (left) striatum that were not visible at t=0min, in particular the anterior striate artery (fig. 1b, long arrow) was very prominent. Immunocytochemistry demonstrated numerous anti-FITC positive vessels in the injected striatum in the territory of the anterior striate artery (fig.1c) whilst no detectable staining was apparent in the contralateral hemisphere (fig. 1d). Animals injected with gd did not exhibit changes in signal intensity on post-gd T_1-weighted images, indicating that the BBB is intact and precluding non-specific entry of the novel contrast agent into the brain tissue.

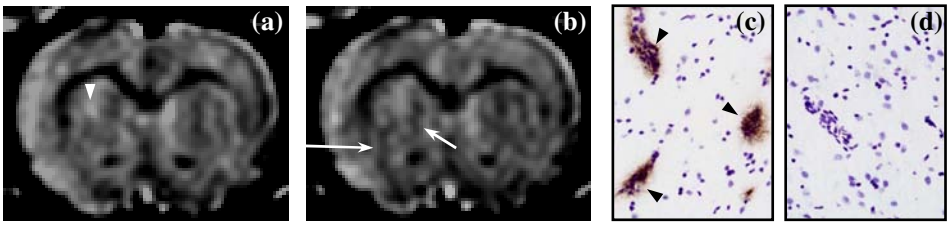

Fig. 1. T_2-weighted images acquired at (a) t=0 and (b) t=30 relative to the LacNAc-USPIO injection. In (a) arrowhead indicates site of IL-1β injection in left striatum. In (b) arrows indicate vessels with reduced signal intensity that are not apparent at t=0. (c) Immunohistochemistry demonstrates positive anti-FITC-staining (brown label [DAB]) of vessels in the left striatum (black arrowheads), but not in the contralateral striatum (d) (x400 original magnification).

Discussion

In this study we have demonstrated that the novel contrast agent LacNAc-CLIO can be used to identify early endothelial activation following intracerebral injection of the proinflammatory cytokine IL-1β In particular, the anterior striate artery and smaller vessels in this vicinity showed strong labeling effects both in the MR images and immunocytochemically. These are vessels that we know from previous studies respond particularly sensitively to the type of intrastriatal challenge used in this study. These data demonstrate the potential of this and other similar targeted contrast agents for the early detection of brain injury and inflammation.

References

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