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Multimodal imaging of subventricular zone neural stem/progenitor cells in the cuprizone mouse model reveals increased neurogenic potential for the olfactory bulb pathway, but no contribution to remyelination of the corpus callosum ☆

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ABSTRACT

Multiple sclerosis is a devastating demyelinating disease of the central nervous system (CNS) in which endogenous remyelination, and thus recovery, often fails. Although the cuprizone mouse model allowed elucidation of many molecular factors governing remyelination, currently very little is known about the spatial origin of the oligodendrocyte progenitor cells that initiate remyelination in this model. Therefore, we here investigated in this model whether subventricular zone (SVZ) neural stem/progenitor cells (NSPCs) contribute to remyelination of the splenium following cuprizone-induced demyelination. Experimentally, from the day of *in situ* NSPC labeling, C57BL/6J mice were fed a 0.2% cuprizone diet during a 4-week period and then left to recover on a normal diet for 8 weeks. Two *in situ* labeling strategies were employed: (i) NSPCs were labeled by intraventricular injection of micron-sized iron oxide particles and then followed up longitudinally by means of magnetic resonance imaging (MRI), and (ii) SVZ NSPCs were transduced with a lentiviral vector encoding the eGFP and Luciferase reporter proteins for longitudinal monitoring by means of *in vivo* bioluminescence imaging (BLI). In contrast to preceding suggestions, no migration of SVZ NSPC towards the demyelinated splenium was observed using both MRI and BLI, and further validated by histological analysis, thereby demonstrating that SVZ NSPCs are unable to contribute directly to remyelination of the splenium in the cuprizone model. Interestingly, using longitudinal BLI analysis and confirmed by histological analysis, an increased migration of SVZ NSPC-derived neuroblasts towards the olfactory bulb was observed following cuprizone treatment, indicative for a potential link between CNS inflammation and increased neurogenesis.

Introduction

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS). Currently, therapeutic interventions are mainly focused on halting or modulating peripheral and CNS immune responses, while only limited research is undertaken towards promoting endogenous repair mechanisms. The latter can be ascribed to the original thoughts that damaged CNS tissue could not be repaired, as neurogenesis was only seen to occur during development. However, due to the discovery of neural stem/progenitor cells (NSPCs) in the adult brain, stimulation of endogenous remyelination processes is emerging as an important therapeutic goal in the treatment of MS (Franklin and Ffrench-Constant, 2008; Menn et al., 2006), as it is well-recognized that endogenous remyelination appears to fail in about 60% of MS lesions (Barkhof et al., 2003). Possible reasons for the latter have most often been studied in animal models for MS. More specifically, the cuprizone mouse model, a toxin-induced murine model for MS without

Abbreviations: AP, antero-posterior; APC/CC1, adenomatous polyposis coli; BG, background; BLI, bioluminescence imaging; CC, corpus callosum; CNS, central nervous system; DV, dorso-ventral; EAE, experimental auto-immune encephalomyelitis; eGFP, enhanced green fluorescent protein; EWD, error weighted difference; fLuc, firefly luciferase; GE, gradient echo; GFAP, glial fibrillary acidic protein; IBA1, ionized calcium binding adaptor molecule; La, lateral; LVv, lentiviral vector; MBP, myelin basic protein; mIPs, minimum intensity projections; MPIO, micron-sized iron oxide particles; MRI, magnetic resonance imaging; MS, multiple sclerosis; NeuN, neuronal nuclear antigen; NSPC, neural stem progenitor cell; OB, olfactory bulb; p75NTR, p75 neurotrophin receptor; p.i., post-injection; PBS, phosphate buffered saline; PC, positive control; PLL, poly-L-lysine; RF, radio-frequency; RMS, rostral migratory stream; ROI, region of interest; sCC, splenium of the corpus callosum; SVZ, subventricular zone; VOI, volume of interest.

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the interference of an ongoing auto-immune inflammatory response, is increasingly used to elucidate factors which contribute to de- and remyelination in MS. During the past decade, the sequence of events during de- and remyelination in the cuprizone model, as well as the accompanying molecular and inflammatory events in the CNS, have been characterized extensively (Kipp et al., 2009). In this model, cuprizone treatment induces selective oligodendrocyte apoptosis resulting in demyelination of the CNS main white matter tracts in the corpus callosum (CC), external capsule and striatum. Moreover, demyelination can also be observed in the cortex and hippocampus, albeit at a later timepoint (Skripuletz et al., 2011).

Following acute cuprizone-induced demyelination (*i.e.* 4–6 weeks of cuprizone treatment), remyelination occurs spontaneously. In this context, Petratos et al. (2004) have demonstrated the presence of a subgroup of $NG2^+$ oligodendroglial progenitor cells (OPCs) in the area surrounding the plaques and in the subventricular zone (SVZ) of MS patients. As the same observation was made in the SVZ and in the CC of mice that were treated with cuprizone for 3 weeks, it was suggested that progenitor cells proliferate in the SVZ and subsequently migrate towards the adjacent CC where they differentiate into mature oligodendrocytes (Petratos et al., 2004). Another study by Mason et al. Mason et al. (2000) clearly demonstrated that cell proliferation in the SVZ occurs before the re-appearance of mature oligodendrocytes in the CC. By performing histological analyses at different time points they initially observed the appearance of $NG2^+$ OPCs in the fornix, after which these $NG2^+$ OPCs appeared to migrate into the overlaying CC (Mason et al., 2000). Additionally, increased proliferation of SVZ NSPCs following 3–6 weeks of cuprizone administration has been reported multiple times by means of histological analysis (Chen et al., 2012; Kipp et al., 2009; Mason et al., 2000). Taken together, this suggests an extreme plasticity of the SVZ NSPCs during injury and repair of the CNS.

Current non-invasive imaging modalities for longitudinal tracking of SVZ NSPC migration include magnetic resonance imaging (MRI) and bioluminescence imaging (BLI) (Couillard-Despres and Aigner, 2011; Couillard-Despres et al., 2011; Vande Velde et al., 2012a). For MRI-based cell tracking, numerous studies reported successful *in situ* labeling of SVZ NSPCs following injection of iron oxide particles in the lateral ventricle (Panizzo et al., 2009; Shapiro et al., 2006; Sumner et al., 2009; Vreys et al., 2010) or the SVZ (Nieman et al., 2010). Although only a minor fraction of the injected iron oxide particles becomes internalized by SVZ NSPCs, migration of SVZ NSPC-derived neuroblasts along the rostral migratory stream (RMS) towards the olfactory bulb (OB) could be monitored longitudinally by means of MRI. Moreover, using this technique, a deviation of the steady state RMS migration pathway was demonstrated for SVZ NSPC-derived cells following a hypoxic–ischemic insult (Yang et al., 2009). For BLI-based cell tracking, transduction of the SVZ with a lentiviral vector (LVv) encoding the firefly luciferase (fLuc) reporter protein has been applied successfully to monitor *in vivo* proliferation and migration of SVZ NSPCs towards the OB (Reumers et al., 2008). Given the advantage of longitudinal assessment of *in vivo* SVZ NSPC behavior over single-time point histological analysis, we applied two non-invasive imaging techniques (MRI and BLI) to study the *in vivo* SVZ NSPC behavior and migration following demyelination in the cuprizone model.

Using this experimental approach, we aimed to investigate: (i) whether or not SVZ NSPC contribute to the spontaneous endogenous remyelination of the splenium that occurs in cuprizone model of CNS de/remyelination, and (ii) whether inflammatory and neurode/regenerative processes occurring in the cuprizone model influence the steady state SVZ NSPC-derived neuroblast migration along the RMS towards the OB. In addition, histological analyses are applied to confirm our non-invasive imaging observations at the study end.

Materials and methods

Animals

Female wild type C57BL/6J mice, 8 weeks of age, were obtained *via* Charles River Laboratories (strain code 027) and used for MRI experiments (n = 16). Female C57BL/6J-*Tyr*$^{c\text{-}2J}$/J mice (albino C57BL/6J mice) were obtained *via* Jackson Laboratories (strain code 000058) and further bred in the animal facility of the University of Antwerp. Female offspring of C57BL/6J-*Tyr*$^{c\text{-}2J}$/J mice, 8 weeks of age, were then used for BLI experiments (n = 20). For all experiments, mice were kept in normal day–night cycle (12/12) with *ad libitum* access to food and water. All experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Antwerp (approval nos. 2006/36 and 2011/13).

Induction of CNS inflammation, demyelination and remyelination

Female C57BL/6J (n = 10) and C57BL/6J-*Tyr*$^{c\text{-}2J}$/J mice (n = 10), 8 weeks of age, had *ad libitum* access to standard rodent lab chow mixed with 0.2% w/w cuprizone (bis(cyclohexanone)oxaldihydrazone, Sigma-Aldrich, Germany) for 4 weeks to induce inflammation and demyelination in the CNS. Following a 4 week 0.2% w/w cuprizone diet, mice were allowed to recover for 8 weeks with *ad libitum* access to standard rodent lab chow. Age matched female control C57BL6 mice (n = 6) and C57BL/6J-*Tyr*$^{c\text{-}2J}$/J mice (n = 10) had *ad libitum* access to standard rodent lab chow during the whole study period.

Preparation of the MPIO–PLL transfection complex

As an MR contrast agent to label SVZ NSPCs, 1.63 μm diameter polystyrene/divinylbenzene-coated fluorescent (Glacial Blue) micron-sized iron oxide particles (MPIOs, 3.00 mg Fe/ml; Bangs Laboratories, Fishers, IN, USA, #ME04F/7833) were used. A cationic polyamine non-viral transfection agent, poly-L-lysine hydrobromide (PLL, MW > 300 kDa, Sigma-Aldrich, Munich, Germany), was used to improve *in situ* cell labeling as previously described (Vreys et al., 2010). Briefly, a PLL stock solution of 1.5 mg/ml was prepared and stored at 5 °C. Prior to injection, the stock solution was further diluted with a 0.9% sodium chloride solution to 0.3 mg/ml and mixed with the MPIOs (3.0 mg Fe/ml), yielding a final MPIO concentration of 0.67 mg Fe/ml with 0.045 mg PLL/ml. MPIO–PLL complexes were placed on a horizontal shaker at 600 rpm for 60 min at room temperature to allow the transfection agent to hybridize with the iron oxide particles. Following preparation, MPIO–PLL complexes were directly used for stereotactic injection.

Lentiviral vector

For genetic labeling of SVZ NSPC we used a previously described LVv encoding both the eGFP and fLuc reporter proteins (LV–eGFP–T2A–fLuc) (Vreys et al., 2010). The LVv was used at a concentration of 2.45×10^8 TU/ml.

Stereotactic injections of MPIO–PLL and LV–eGFP–T2A–fLuc

All surgical interventions were performed according to institutional guidelines. Briefly, mice were anesthetized by intraperitoneal (IP) injection of a ketamine (50 mg/kg for MPIO–PLL injection and 75 mg/kg for LVv injection) (Anesketin; Eurovet NV/SA, Heusden-Zolder, Belgium) + medetomidine (0.65 mg/kg for MPIO–PLL injection and 1 mg/kg for LVv injection) (Domitor; Pfizer Animal Health S.A., Louvain-la-Neuve, Belgium) mixture and positioned in a stereotactic head frame. Stereotactic coordinates to target the right lateral ventricle for MPIO–PLL injection were as follows: AP 1.0 mm, La 0.75 mm and DV 3.3 mm (relative to the bregma). Stereotactic coordinates to target