Neuroscience Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Short communication

Ferumoxytol administration does not alter infarct volume or the inflammatory response to stroke in mice

Kristian P. Doyle[a,b], Lisa N. Quach[a], Helen E.D' Arceuil[c], Marion S. Buckwalter[a,d,*]

[a] Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA
[b] Department of Immunobiology, Department of Neurology, and the Arizona Center on Aging, University of Arizona, Tucson, AZ, USA
[c] Department of Radiology, Stanford University School of Medicine, Stanford, CA, USA
[d] Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA

HIGHLIGHTS

- Ferumoxytol is an ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle.
- Ferumoxytol is beginning to be used off-label as an imaging agent in stroke.
- USPIOs similar to ferumoxytol can activate peripheral macrophages.
- Ferumoxytol did not alter infarct volume or the inflammatory response to stroke.
- Ferumoxytol as a contrast agent does not adversely affect stroke outcome in mice.

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form 16 October 2014
Accepted 23 October 2014
Available online xxx

Keywords:
Stroke
Ferumoxytol
Magnetic resonance imaging
Inflammation

ABSTRACT

Ferumoxytol is an ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle that is FDA-approved as an intravenous iron replacement therapy for the treatment of iron deficiency anemia in patients with chronic kidney disease. Ferumoxytol has also been used as a contrast agent for cerebral blood volume mapping by magnetic resonance imaging (MRI), which suggests it could be used for imaging hemodynamic abnormalities after stroke. However, circulating macrophages can internalize USPIOs, and recent data indicate that the accumulation of iron in macrophages can lead them to adopt the M1 pro-inflammatory phenotype. Therefore, the uptake of intravenously administered iron particles by circulating macrophages that home to the stroke core could potentially alter the inflammatory response to stroke. To test this possibility *in vivo* we administered a dose of ferumoxytol previously used to obtain cerebral blood volume maps in healthy humans by steady-state susceptibility contrast (SSC) MRI to BALB/cJ mice 48 h after stroke and examined cytokine levels, microglial/macrophage activation, and lesion volume in the brain 5 days later. Treatment with ferumoxytol did not lead to any differences in these parameters. These data indicate that the use of ferumoxytol as a contrast agent for brain imaging after stroke does not alter the inflammatory response to stroke in mice, and is therefore unlikely to do so in human subjects.

1. Introduction

Ferumoxytol is an ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle that is FDA-approved as an intravenous iron replacement therapy for the treatment of iron deficiency anemia in patients with chronic kidney disease. Ferumoxytol has also been used for MRI cerebral blood volume mapping (CBV) and cellular imaging of inflammation in the injured CNS [4,22].

Currently, the primary method for cerebral blood volume mapping is to use dynamic susceptibility contrast (DSC) MRI following bolus administration of gadolinium-based contrast agents. However, this technique requires a fast image acquisition protocol, which limits spatial resolution [2,3]. Although ferumoxytol can also be used for DSC MRI, recently, ferumoxytol has been used to generate higher resolution CBV maps in humans by steady-state susceptibility contrast perfusion mapping (SS CBV) [3,21].

* Corresponding author at: Departments of Neurology and Neurological Sciences and Neurosurgery, Stanford University Medical School 1201 Welch Road, Stanford, CA, 94305, USA. Tel.: +1 650 724 9098.
E-mail address: marion.buckwalter@stanford.edu (M.S. Buckwalter).

http://dx.doi.org/10.1016/j.neulet.2014.10.041
0304-3940/

Ferumoxytol can be used in this way because it has an intravascular half-life of ~12 h in humans [3], which contrasts with a typical intravascular half-life of 1.6 h for gadolinium-based contrast agents [22]. Therefore, ferumoxytol could be a useful contrast agent for perfusion mapping after stroke.

In addition, USPIOs such as ferumoxytol may be useful to image inflammation after stroke [24]. USPIOs can be ingested by activated peripheral monocytes and macrophages that home to the injured brain to participate in the clearance of dead cells and cellular debris after stroke [1,8,25]. Ingestion by macrophages occurs *via* both receptor-mediated endocytosis and phagocytosis [9,16], both of which are processes that activate intracellular signaling pathways. This raises the possibility that prior ferumoxytol uptake could alter the way macrophages respond to inflammation in the brain after stroke. Indeed, in support of this possibility, Sindrilaru et al. recently showed that uptake of iron by cells of the mononuclear phagocyte system can cause them to adopt a pro-inflammatory M1 phenotype [20]. In addition, iron oxide particles of various sizes and with diverse coatings have been reported to have varying effects on macrophages *in vitro*, including pro-inflammatory, anti-inflammatory, and no effects [10,13,14,19]. For ferumoxytol itself there is no available data on its effects on macrophage function.

Therefore, to test if the administration of ferumoxytol to stroke patients might alter their inflammatory responses and change stroke outcomes, we sought to test its effects on the immune response to stroke in mice. We injected wildtype BALB/cJ mice 2 days after experimental stroke with a ferumoxytol dose equivalent to that used for SS CBV in humans. We compared final (5 days post-injection) lesion size and the attendant inflammatory response to stroke between mice that received ferumoxytol and vehicle control injections.

2. Methods

Mice: We used 45 12-week-old male BALB/cJ mice for this study. All procedures met NIH guidelines with the approval of the Stanford University Institutional Animal Care and Use Committee.

Stroke surgery and ferumoxytol treatment: Distal middle cerebral artery occlusion (DMCAO) was induced as described [5]. Briefly, animals were anesthetized and the middle cerebral artery (MCA) exposed and cauterized. Mice were injected *via* the tail vein with ferumoxytol (Feraheme, AMAG Pharmaceuticals, Inc., Cambridge, MA) at a concentration of 7 mg/kg, or saline vehicle, 48 h after DMCAO. Mice were euthanized 7 days post DMCAO for elucidation of infarct volume and immunostaining ($n = 9$ per group) and multiplex immunoassay ($n = 11–12$ per group).

Immunohistochemistry and infarct volume assessment: Immunostaining and infarct assessment were performed using standard techniques on PFA-fixed 40 μm coronal brain sections. Microglial/macrophage immunostaining was performed using an anti-CD68 antibody (Serotec #MCA1957). To calculate stroke volume as a percentage of the contralateral side, combined cresyl violet and NeuN (Millipore #MAB377B) histology was used to trace the non-infarcted ipsilateral hemisphere and contralateral hemisphere in six sections per mouse spaced 640 μm apart using ImageJ software.

Multiplex immunoassay: After saline perfusion, the lesion (stroke core) was dissected, immersed in lysis buffer (Sigma Cel-Lytic MT Cell Lysis Reagent containing Sigma protease inhibitor cocktail and Sigma phosphatase inhibitor cocktail 2) at a ratio of 1:20, and sonicated. Cytokines and chemokines were detected and quantified in duplicate by multiplex immunoassay performed by the Human Immune Monitoring Center at Stanford University (himc.stanford.edu).

Perls' iron staining: A Perls' iron stain, a 1:1 mixture of 10% $K_4Fe(CN)_6$ and 20% HCl solution, was performed in conjunction with anti-CD68 immunostaining on six coronal brain sections per mouse spaced 640 μm apart. To calculate the percentage of sections with Perls' iron staining, photomicrograph images were taken of four regions of interest from each section in an unbiased, blinded, fashion. The regions of interest were the cortex adjacent to the lesion, the stroke border, the stroke core, and the ipsilateral side of the corpus callosum. Each image was assessed for the presence or absence of iron staining, and for each region the number of images with observable iron staining was divided by the total number of images.

Statistical analysis: Allocation of mice into groups was performed randomly. Data are expressed as means ± standard error of the mean (SEM) unless otherwise indicated in the figure legend. Statistical analyses were performed with Prism 5 software (GraphPad-San Diego, CA). Means between two groups were compared with a two-tailed, unpaired Student's *t*-test for cytokines, and Mann–Whitney test for iron staining.

3. Results

3.1. Administration of ferumoxytol does not alter cytokine expression in the stroke lesion

The induction of pro-inflammatory cytokines and chemokines in the stroke lesion contributes to secondary injury after stroke [6]. To investigate whether the administration of ferumoxytol after DMCAO leads to altered expression of cytokines and chemokines in the stroke lesion, ferumoxytol (7 mg/kg) or saline was intravenously administered *via* the tail vein to BALB/cJ mice 48 h after DMCAO. Mice were euthanized 5 days later and the stroke lesion was dissected and used for a multiplex immunoassay. Ferumoxytol administration did not lead to any significant changes in the expression of MCP1, MCP3, MIP1A, Eotaxin, GMCSF, GCSF, IL1A, IL2, IL5, IL6, IL10, IFNγ, TNFα, IL12p40, IL12p70, IP10, RANTES, TGFβ, or VEGF (Fig. 1). Levels of IL1β, IL3, IL4, IL17, IL23p19, KC, and IL13 were undetectable in the stroke lesion in both the ferumoxytol- and vehicle-treated mice 7 days after stroke (data not shown).

3.2. Administration of ferumoxytol does not alter CD68 expression in the stroke lesion and penumbra

Next we performed immunostaining for CD68 to determine if administration of ferumoxytol alters the monocytic response to stroke, which is composed of both activated brain resident microglia and macrophages that have migrated into the stroke core from the bloodstream [17,18]. CD68 is a lysosome membrane glycoprotein, and antibodies against CD68 are widely used to assess microglial/macrophage activation after stroke [5]. Administration of ferumoxytol did not lead to any alteration in the % area covered by CD68 immunoreactivity (Fig. 2A and B), suggesting that ferumoxytol administration does not change microglial or macrophage activation in the area of injury. Ferumoxytol administration also did not lead to any overt differences in microglial/macrophage morphology, with cells from mice in both treatment groups retaining a similar amoeboid shape and distribution in the stroke lesion and penumbra (Fig. 2C).

3.3. Effect of ferumoxytol administration on brain iron content

To determine if the dose of ferumoxytol required for SS CBV leads to a prolonged increase in iron content in the stroked brain, a Perls' iron stain was performed 7 days after stroke, 5 days after ferumoxytol administration. Overall, Perls' iron staining was similar between saline- and ferumoxytol-injected mice. Brain sections

Please cite this article in press as: K.P. Doyle, et al., Ferumoxytol administration does not alter infarct volume or the inflammatory response to stroke in mice, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.10.041

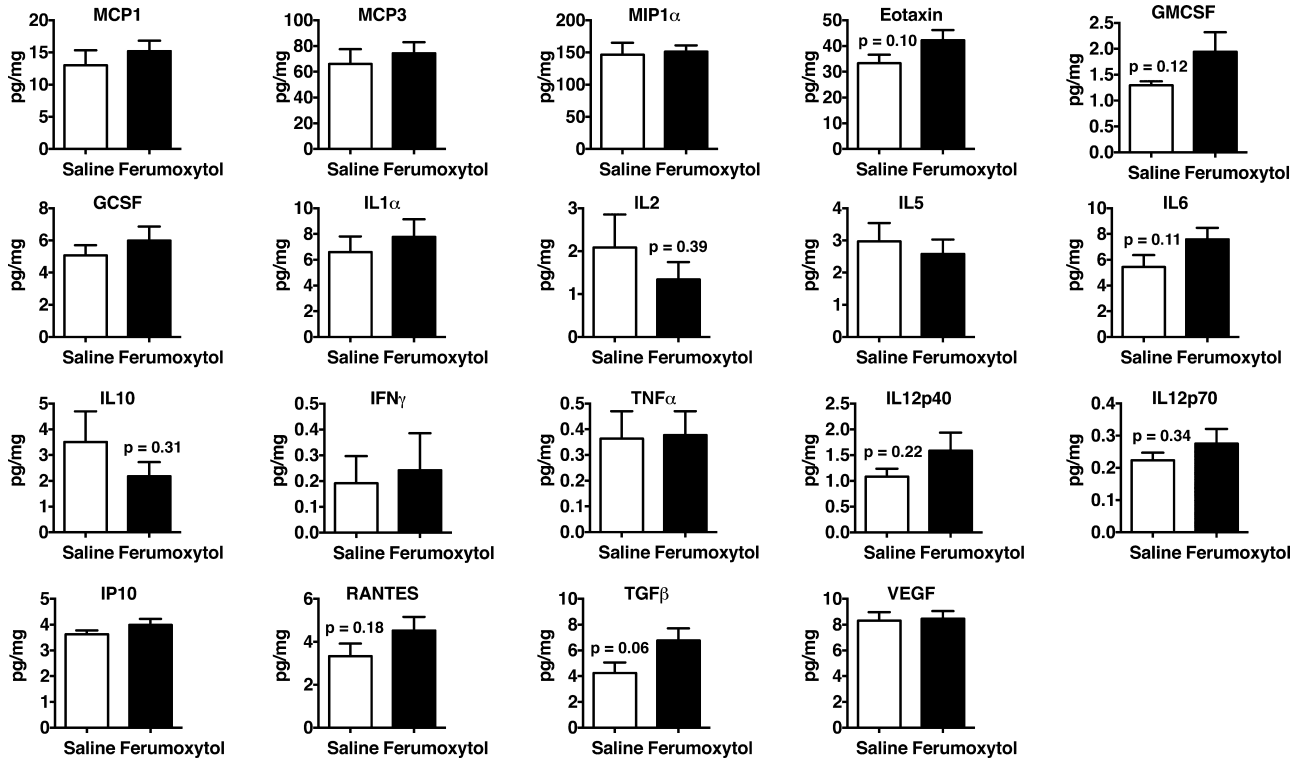

Fig. 1. Administration of ferumoxytol does not alter cytokine or chemokine expression 7 days after stroke. Graphs showing cytokine and chemokine levels in the stroke lesion 5 days post ferumoxytol or saline treatment. There were no significant ($P < 0.05$) differences between treatment groups ($n = 11$ for saline group, $n = 12$ for ferumoxytol group).

contained either no staining or only sparse amounts of iron, and the large majority of CD68+ cells did not co-localize with Perls' staining. When Perls' staining was seen it was typically associated with areas of macrophage infiltration and activation, as defined by CD68+ immunostaining (Fig. 3A). This result suggests that the majority of the iron present in the lesion is derived from blood that was trapped in the lesion at the time of the stroke, which is a permanent occlusion model, or from microhemorrhages after stroke. The only difference observed was rare, but significantly more, iron staining in the corpus callosum underlying and medial to the lesion in ferumoxytol-injected mice (Fig. 3B and C). We confirmed that this was increased after ferumoxytol injection by scoring the brain regions with staining. The percent of sections with staining was not different in the cortex, stroke core, or border, but

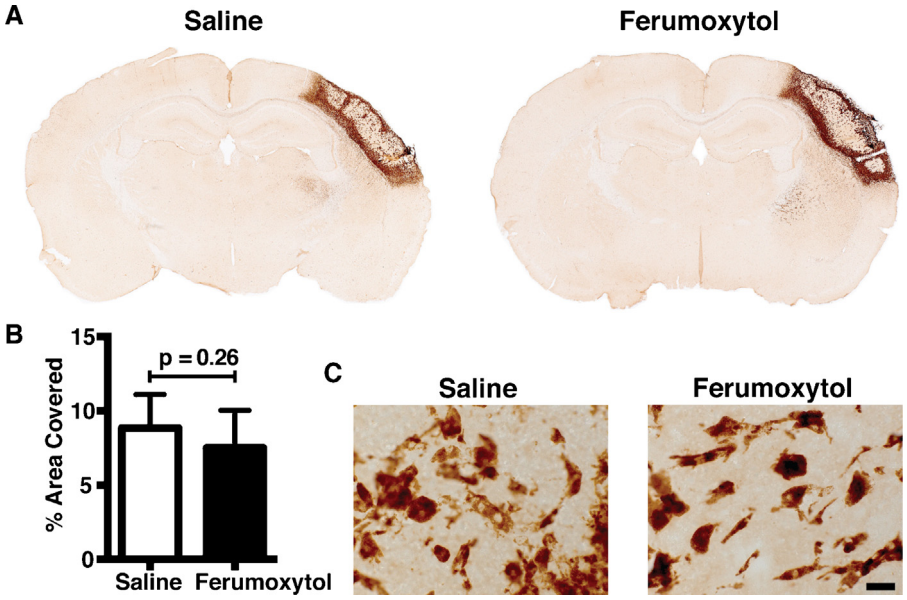

Fig. 2. Administration of ferumoxytol does not alter CD68 immunoreactivity 7 days after stroke. (A) Whole section images showing CD68 immunoreactivity in the lesion and in areas of axonal degeneration 5 days after saline and ferumoxytol administration and 7 days after DMCAO. (B) There is no difference in % area covered by CD68 immunoreactivity in mice that received ferumoxytol ($n = 9$ per group). (C) There is also no overt difference in CD68$^+$ cell morphology in mice that received ferumoxytol. Error bars are SD, scale bar: 50 μm.

Please cite this article in press as: K.P. Doyle, et al., Ferumoxytol administration does not alter infarct volume or the inflammatory response to stroke in mice, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.10.041

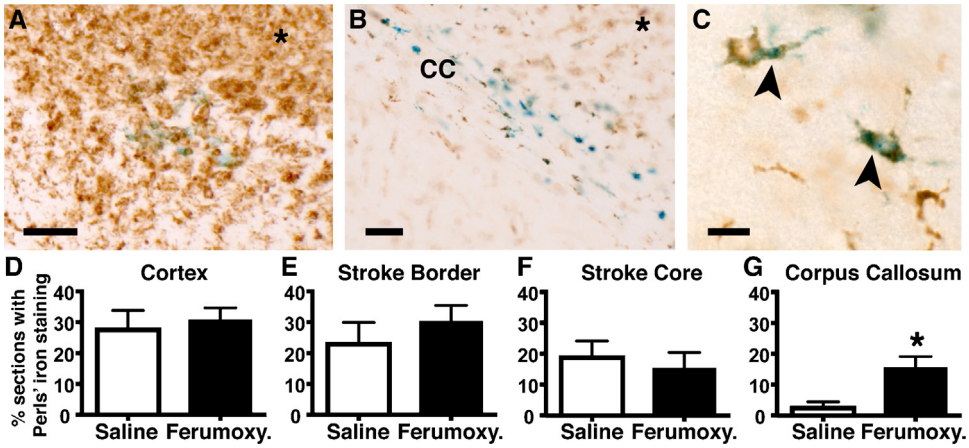

Fig. 3. Ferumoxytol administration leads to increased Perls' iron staining in the corpus callosum but not in peri-infarct regions. (A) Representative image of iron staining (blue) among macrophages (brown) in the stroke core (asterisk) and border. Scale bar: 50 μm. (B) Low magnification image of iron staining in the corpus callosum underlying the stroke (asterisk). Scale bar: 50 μm. (C) Higher magnification image of co-localization (marked by arrowheads) of Perls' iron staining in CD68-expressing activated microglia or macrophages in the corpus callosum adjacent to the stroke. Scale bar: 10 μm. (D–G) Quantification of the percentage of sections per mouse with iron staining in the cortex ($p = 0.76$), stroke border ($p = 0.49$), stroke core ($p = 0.58$), and corpus callosum ($^*p = 0.03$). (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article).

was increased in the corpus callosum (Fig. 3D–G). This indicates that the dose of ferumoxytol that has been successfully used for SS CBV in humans does cause loading of ferumoxytol in scattered cells that localize to the perilesional area but does not markedly alter the long-term iron load in the stroked brain.

3.4. Ferumoxytol administration did not alter infarct volume after stroke

To determine if ferumoxytol administration 48 h after stroke has an effect on infarct volume, brain sections were stained with cresyl violet. To provide increased contrast between infarcted and non-infarcted tissue, sections were counterstained with an antibody against the neuronal nuclear antigen NeuN. Combined cresyl violet and NeuN histology revealed that lesion size 7 days following stroke is equivalent in mice administered ferumoxytol and mice administered the vehicle control (Fig. 4). This provides evidence that intravascular administration of the dose of ferumoxytol sufficient for SS CBV mapping in humans (7 mg/kg) does not lead to increased injury when administered 48 h after stroke.

4. Discussion

The purpose of this study was to ask whether or not the use of ferumoxytol for SS CBV mapping after stroke can alter the inflammatory response to stroke and thereby affect stroke outcome. Ferumoxytol nanoparticles are iron oxide coated with polyglucose sorbitol carboxymethylether. Ferumoxytol has a number of properties that make it desirable as a contrast agent for perfusion studies. The carbohydrate outer coating reduces immunogenicity and enhances iron retention [15], the nanoparticles have a diameter of 17–31 nm, which confines them to the intravascular space [11], and ferumoxytol has a half-life in the circulation of approximately 12 h, which means it can be used for SS CBV MRI to generate maps with an isotropic spatial resolution of up to 1 mm^3 [3]. This spatial resolution makes ferumoxytol an excellent candidate contrast agent for visualizing small, variegated, and complex lesions after stroke.

Following intravascular administration, ferumoxytol nanoparticles are cleared by mononuclear phagocytes in the spleen and liver, but prior to clearance, a small but significant proportion of ferumoxytol can be taken up by circulating activated mononuclear phagocytes [4,22]. Sindrilaru et al. demonstrated that iron uptake by mononuclear phagocytes can cause cells to adopt a pro-inflammatory M1 phenotype [20], and because mononuclear cells migrate to the stroke, the administration of ferumoxytol could make the inflammatory response to stroke more damaging. To test this possibility we administered a dose of ferumoxytol that has been validated for SS CBV MRI (7 mg/kg) at 2 days post-stroke, a time point when perfusion mapping after stroke is clinically relevant, and looked at post stroke inflammation and lesion size 5 days later, when macrophage infiltration reaches its peak [6].

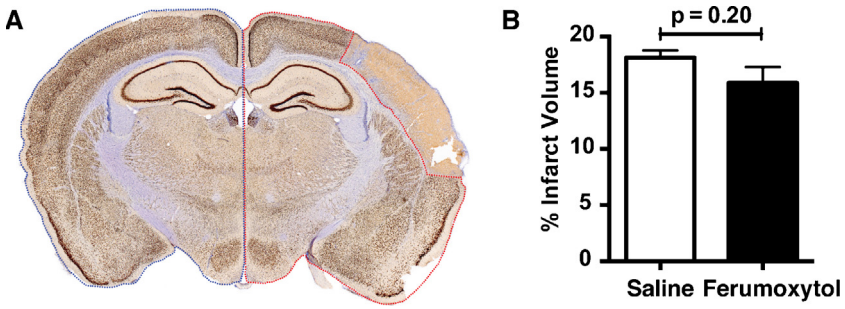

Fig. 4. Lesion size is not altered 5 days after administration of ferumoxytol compared to after administration of saline. (A) Representative whole section image showing cresyl violet staining and NeuN immunoreactivity 7 days after DMCAO. These sections were used to calculate infarct volume. The area of the ipsilateral hemisphere excluding the stroke core (outlined in red) was subtracted from the area of the contralateral hemisphere (outlined in blue) and then divided by the area of the contralateral hemisphere to calculate infarct volume as a percentage of the contralateral hemisphere. (B) There is no difference in % infarct volume between saline- and ferumoxytol-treated mice ($n = 9$ per group). (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article).

Please cite this article in press as: K.P. Doyle, et al., Ferumoxytol administration does not alter infarct volume or the inflammatory response to stroke in mice, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.10.041

In this experimental paradigm, ferumoxytol did not alter the inflammatory response to stroke, as assessed by cytokine expression and CD68 immunoreactivity, or have an impact on lesion size. This suggests that 7 mg/kg of ferumoxytol does not cause monocytes/macrophages that migrate to the brain after stroke to adopt a more pro-inflammatory phenotype than otherwise engendered by the stroke. This finding is not entirely unexpected. In contrast to the 7 mg/kg of ferumoxytol we used here, Sindrilaru et al. administered 1400 mg/kg of iron-dextran to each mouse in their study, a substantially greater quantity of iron. Additionally, in a previous study, when 25 μg of ferumoxytol was injected directly into the brains of rats, it did not cause any overt signs of toxicity three months later [12]. These data suggest that while iron overloading can cause macrophages to adopt a pro-inflammatory M1 phenotype, the dose of ferumoxytol used for SS CBV MRI does not lead to changes in the inflammatory state of macrophages.

We initially hypothesized that 7 mg/kg of ferumoxytol would preload a significant number of circulating monocytes/macrophages that would then infiltrate the stroke and lead to an increase in iron staining throughout the infarcted region of the brain. There was an increase in the amount of staining in the corpus callosum under the stroke, so some preloaded macrophages likely do infiltrate the brain after this dose of ferumoxytol. However, we did not find increased Perls' staining within, or at the border of the stroke lesion in the brains of mice that were treated with ferumoxytol compared to control mice, and indeed most of the macrophages in the stroke core did not exhibit Perls' iron staining.

The relative paucity of staining could be due to ferumoxytol not being efficiently taken up by circulating mononuclear cells. This is supported by a rat study, in which only 0.5% of circulating mononuclear cells were positive for iron two hours following intravenous injection of ferumoxytol and protamine sulfate at a dose of 20 mg/kg of iron [25]. However, ferumoxytol has an intravascular half-life of approximately 12 h in humans, 80 min in rats, and 45 min in mice [7,8,23]. This means that the longer half-life of ferumoxytol in the human circulation could lead to more iron preloading of macrophages in humans than occurs in mice.

In conclusion, although our data is restricted to mice, this study provides evidence that the administration of ferumoxytol at a dose sufficient for perfusion mapping after stroke does not cause significant preloading of peripheral monocytes/macrophages or affect the inflammatory response to stroke. We interpret this to mean that ferumoxytol administration at doses sufficient for perfusion mapping are unlikely to affect stroke outcomes. The impact of higher doses is unknown.

Acknowledgement

This work was funded by NIH, K99NR013593 (KPD), NS47607 (HDA), and R21NS078571 (MSB).

References

[1] A.S. Arbab, B. Janic, J. Haller, E. Pawelczyk, W. Liu, J.A. Frank, In vivo cellular imaging for translational medical research, Curr. Med. Imaging Rev. 5 (2009) 19–38.
[2] D.J. Atkinson, D. Burstein, R.R. Edelman, First-pass cardiac perfusion: evaluation with ultrafast MR imaging, Radiology 174 (1990) 757–762.
[3] T. Christen, W. Ni, D. Qiu, H. Schmiedeskamp, R. Bammer, M. Moseley, G. Zaharchuk, High-resolution cerebral blood volume imaging in humans using the blood pool contrast agent ferumoxytol, Magn. Reson. Med. (2012).
[4] E. Dosa, S. Tuladhar, L.L. Muldoon, B.E. Hamilton, W.D. Rooney, E.A. Neuwelt, MRI using ferumoxytol improves the visualization of central nervous system vascular malformations, Stroke 42 (2011) 1581–1588.
[5] K.P. Doyle, E. Cekanaviciute, L.E. Mamer, M.S. Buckwalter, TGFbeta signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke, J. Neuroinflammation 7 (2010) 62.
[6] N.V. Gronberg, F.F. Johansen, U. Kristiansen, H. Hasseldam, Leukocyte infiltration in experimental stroke, J. Neuroinflammation 10 (2013) 115.
[7] C. Harms, A.L. Datwyler, F. Wiekhorst, L. Trahms, R. Lindquist, E. Schellenberger, S. Mueller, G. Schutz, F. Roohi, A. Ide, M. Fuchtemeier, K. Gertz, G. Kronenberg, U. Harms, M. Endres, U. Dirnagl, T.D. Farr, Certain types of iron oxide nanoparticles are not suited to passively target inflammatory cells that infiltrate the brain in response to stroke, J. Cereb. Blood Flow Metab. 33 (2013) e1–9.
[8] D.M. Hasan, K.B. Mahaney, V.A. Magnotta, D.K. Kung, M.T. Lawton, T. Hashimoto, H.R. Winn, D. Saloner, A. Martin, S. Gahramanov, E. Dosa, E. Neuwelt, W.L. Young, Macrophage imaging within human cerebral aneurysms wall using ferumoxytol-enhanced MRI: a pilot study, Arterioscler Thromb Vasc. Biol. 32 (2012) 1032–1038.
[9] E.C. Henning, C.A. Ruetzler, M.R. Gaudinski, T.C. Hu, L.L. Latour, J.M. Hallenbeck, S. Warach, Feridex preloading permits tracking of CNS-resident macrophages after transient middle cerebral artery occlusion, J. Cereb. Blood Flow Metab. 29 (2009) 1229–1239.
[10] J.K. Hsiao, H.H. Chu, Y.H. Wang, C.W. Lai, P.T. Chou, S.T. Hsieh, J.L. Wang, H.M. Liu, Macrophage physiological function after superparamagnetic iron oxide labeling, NMR Biomed. 21 (2008) 820–829.
[11] M. Lu, M.H. Cohen, D. Rieves, R. Pazdur, FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease, Am. J. Hematol. 85 (2010) 315–319.
[12] L.L. Muldoon, M. Sandor, K.E. Pinkston, E.A. Neuwelt, Imaging, distribution, and toxicity of superparamagnetic iron oxide magnetic resonance nanoparticles in the rat brain and intracerebral tumor, Neurosurgery 57 (2005) 785–796, discussion 785–796.
[13] K. Muller, J.N. Skepper, M. Posfai, R. Trivedi, S. Howarth, C. Corot, E. Lancelot, P.W. Thompson, A.P. Brown, J.H. Gillard, Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro, Biomaterials 28 (2007) 1629–1642.
[14] R.D. Oude Engberink, S.M. van der Pol, E.A. Dopp, H.E. de Vries, E.L. Blezer, Comparison of SPIO and USPIO for in vitro labeling of human monocytes: MR detection and cell function, Radiology 243 (2007) 467–474.
[15] A.B. Pai, A.O. Garba, Ferumoxytol: a silver lining in the treatment of anemia of chronic kidney disease or another dark cloud? J. Blood Med. 3 (2012) 77–85.
[16] I. Raynal, P. Prigent, S. Peyramaure, A. Najid, C. Rebuzzi, C. Corot, Macrophage endocytosis of superparamagnetic iron oxide nanoparticles: mechanisms and comparison of ferumoxides and ferumoxtran-10, Invest. Radiol. 39 (2004) 56–63.
[17] M. Schilling, M. Besselmann, M. Muller, J.K. Strecker, E.B. Ringelstein, R. Kiefer, Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice, Exp. Neurol. 196 (2005) 290–297.
[18] M. Schroeter, S. Jander, I. Huitinga, O.W. Witte, G. Stoll, Phagocytic response in photochemically induced infarction of rat cerebral cortex. The role of resident microglia, Stroke 28 (1997) 382–386.
[19] I. Siglienti, M. Bendszus, C. Kleinschnitz, G. Stoll, Cytokine profile of iron-laden macrophages: implications for cellular magnetic resonance imaging, J. Neuroimmunol. 173 (2006) 166–173.
[20] A. Sindrilaru, T. Peters, S. Wieschalka, C. Baican, A. Baican, H. Peter, A. Hainzl, S. Schatz, Y. Qi, A. Schlecht, J.M. Weiss, M. Wlaschek, C. Sunderkotter, K. Scharffetter-Kochanek, An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice, J. Clin. Invest. 121 (2011) 985–997.
[21] C.G. Varallyay, E. Nesbit, R. Fu, S. Gahramanov, B. Moloney, E. Earl, L.L. Muldoon, X. Li, W.D. Rooney, E.A. Neuwelt, High-resolution steady-state cerebral blood volume maps in patients with central nervous system neoplasms using ferumoxytol, a superparamagnetic iron oxide nanoparticle, J. Cereb. Blood Flow Metab. 33 (2013) 780–786.
[22] J.S. Weinstein, C.G. Varallyay, E. Dosa, S. Gahramanov, B. Hamilton, W.D. Rooney, L.L. Muldoon, E.A. Neuwelt, Superparamagnetic iron oxide nanoparticles: diagnostic magnetic resonance imaging and potential therapeutic applications in neurooncology and central nervous system inflammatory pathologies, a review, J. Cereb. Blood Flow Metab. 30 (2010) 15–35.
[23] R. Weissleder, G. Elizondo, J. Wittenberg, C.A. Rabito, H.H. Bengele, L. Josephson, Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging, Radiology 175 (1990) 489–493.
[24] M. Wiart, N. Davoust, J.B. Pialat, V. Desestret, S. Moucharrafie, T.H. Cho, M. Mutin, J.B. Langlois, O. Beuf, J. Honnorat, N. Nighoghossian, Y. Berthezene, MRI monitoring of neuroinflammation in mouse focal ischemia, Stroke 38 (2007) 131–137.
[25] Y.J. Wu, L.L. Muldoon, C. Varallyay, S. Markwardt, R.E. Jones, E.A. Neuwelt, In vivo leukocyte labeling with intravenous ferumoxides/protamine sulfate complex and in vitro characterization for cellular magnetic resonance imaging, Am. J. Physiol. Cell Physiol. 293 (2007) C1698–C1708.

Please cite this article in press as: K.P. Doyle, et al., Ferumoxytol administration does not alter infarct volume or the inflammatory response to stroke in mice, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.10.041