Intravenous transplantation of mouse embryonic stem cells attenuates demyelination in an ICR outbred mouse model of demyelinating diseases

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How to cite this article: Pringproa K, Sathanawongs A, Khamphilai C, Sukkarinprom S, Oranratnachai A (2016) Intravenous transplantation of mouse embryonic stem cells attenuates demyelination in an ICR outbred mouse model of demyelinating diseases. Neural Regen Res 11(10):1603-1609.

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Funding: This study was supported by the Faculty Research Fund, Faculty of Veterinary Medicine, Chiang Mai University, Thailand.

Abstract

Induction of demyelination in the central nervous system (CNS) of experimental mice using cuprizone is widely used as an animal model for studying the pathogenesis and treatment of demyelination. However, different mouse strains used result in different pathological outcomes. Moreover, because current medicinal treatments are not always effective in multiple sclerosis patients, so the study of exogenous cell transplantation in an animal model is of great importance. The aims of the present study were to establish an alternative ICR outbred mouse model for studying demyelination and to evaluate the effects of intravenous cell transplantation in the present developed mouse model. Two sets of experiments were conducted. Firstly, ICR outbred and BALB/c inbred mice were fed with 0.2% cuprizone for 6 consecutive weeks; then demyelinating scores determined by luxol fast blue stain or immunolabeling with CNPase were evaluated. Secondly, attenuation of demyelination in ICR mice by intravenous injection of mES cells was studied. Scores for demyelination in the brains of ICR mice receiving cell injection (mES cells-injected group) and vehicle (sham-inoculated group) were assessed and compared. The results showed that cuprizone significantly induced demyelination in the cerebral cortex and corpus callosum of both ICR and BALB/c mice. Additionally, intravenous transplantation of mES cells potentially attenuated demyelination in ICR mice compared with sham-inoculated groups. The present study is among the earliest reports to describe the cuprizone-induced demyelination in ICR outbred mice. Although it remains unclear whether mES cells or trophic effects from mES cells are the cause of enhanced remyelination, the results of the present study may shed some light on exogenous cell therapy in central nervous system demyelinating diseases.

Key Words: nerve regeneration; ICR outbred mice; cuprizone; demyelination; embryonic stem cells; remyelination; immunohistochemistry; neural regeneration

Introduction

Demyelination in the central nervous system (CNS) is known to be one of the important pathological features of multiple sclerosis (MS) in humans (Frohman et al., 2006; Rodriguez, 2007). MS is a complex multifactorial disease whose exact pathophysiology remains incompletely explained (Frohman et al., 2006; Dendrou et al., 2015). Until recently, several animal models have been utilized for the study of MS (Gao et al., 2000; Kipp et al., 2009; Koutsoudaki et al., 2009, 2010; Crocker et al., 2011). However, all of the established animal models only partly mimic the processes of MS, with each model having its own advantages and disadvantages (Salinas Tejedor et al., 2015). In addition, although several therapeutic treatments for MS using animal models have been investigated, the results are still uncertain and largely controversial (Crocker et al., 2011; El-Akabawy and Rashed, 2015; Salinas Tejedor et al., 2015). Thus, seeking alternative animal models and therapeutic strategies is essential in order to further enhance our understanding of the complex mechanisms involved in this disease.

Cuprizone (bis-cyclohexanone-oxaldihydrazone) is a copper-chelating agent, and has long been known for its potent induction of CNS demyelination (Ludwin, 1978; Cammer, 1999; Skripuletz et al., 2011). Continued ingestion of cuprizone results in selective death of oligodendrocytes and consequent demyelination in white matter, a pathology which resembles that of pattern III MS lesions (Steelman et al., 2012). The effects of cuprizone toxicity have previously been investigated in several animal models, including mice, rats, guinea pigs, and hamsters (Adamo et al., 2006; Skripuletz et al., 2008; Kanno et al., 2012; Basoglu et al., 2013). In mice, most of the studies have been performed in inbred mouse strains, including Swiss mice, C57BL/6, BALB/c, Albino, CD1, BSVS, and SJL mice (Ludwin, 1978; Hiremath et...
al., 1998; Skripuletz et al., 2008; Praet et al., 2014). However, the clinical course and severity of demyelination in the CNS by cuprizone vary considerably, depending on the gender, age, and strain of mouse (Skripuletz et al., 2010; Praet et al., 2014). It remains to be determined whether an outbred mouse stock would show clinical and pathological lesions resembling those of inbred mice. Moreover, it would be of interest to verify whether an outbred mouse stock could be used as an alternative animal model for studying demyelination and remyelination in the CNS.

Studies of remyelination in the CNS using animal models are largely based on exogenous cell transplantation (Einstein et al., 2009; Crocker et al., 2011; Nessler et al., 2013; El-Akabawy and Rashed, 2015; Glenn et al., 2015). Although several previous studies on transplantation of either mesenchymal or embryonic stem cells have reported improved clinical scores for remyelination in the CNS, most of these studies were performed using inbred mouse strains (Hedayatpour et al., 2013; El-Akabawy and Rashed, 2015; Glenn et al., 2015). It is known that the genetic backgrounds of inbred and outbred mice are different (Chia et al., 2005). Inbred strains offer a defined genetic background, while outbred stocks provide a diverse gene pool, which is closer to the situation in most human studies (Vaickus et al., 2010). Thus, it is worthwhile determining whether outbred mice could be used as an alternative mouse strain of demyelinating disease. To investigate this, the present study aimed (1) to investigate whether demyelination can be induced in outbred ICR mice using cuprizone, and, if yes, (2) to use this animal model to investigate the remyelination potential of mES cells following intravenous transplantation. The results obtained in this study may shed some light on the use of exogenous cell therapy in human demyelinating diseases.

Materials and Methods

Animals

Thirty-five, 7–8-week-old, male ICR outbred and BALB/c inbred mice weighing 30–45 g were purchased from the National Laboratory Animal Center, Mahidol University, Thailand, and housed under strict hygienic conditions at the animal facilities at the Faculty of Veterinary Medicine, Chiang Mai University, Thailand. Upon arrival, animals were separated into groups and acclimatized in cages in a controlled environment (22.5 ± 1°C, 55 ± 5% relative humidity, 12-hour light/dark cycle). The study was conducted in accordance with the United States National Institute of Health Guide for the Care and Use of Laboratory Animals, and all experiments were approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University (permission number: FVM-ACUC.R16/2555).

Preparation of mouse embryonic stem (mES) cells

Mouse embryonic stem (mES) cells derived from B6D2ZF1 mice were grown and maintained at the Center of Biotechnology, Chiang Mai, Thailand, as previously described (Doungpunta et al., 2009). Briefly, cells were grown in knockout Dulbecco’s Modified Eagle’s Medium (DMEM), 2 mM L-glutamine, 0.1 mM MEM non-essential amino acid solution, 0.1 mM 2-beta mercaptoethanol, leukemia inhibitory factor (LIF), and 20% knockout serum replacement. Phenotypic characterization of mES cells was done by immunostaining with alkaline phosphatase and octamer-4 (Doungpunta et al., 2009). Prior to transplantation, cells were washed twice with phosphate buffered saline (PBS), pH 7.2, detached by mechanical shaking, and resuspended in DMEM at a density of 5 × 10^5 cells/µL.

Experimental designs and transplantation protocol

In the first set of experiments, induction of demyelination in ICR and BALB/c mice (n = 6 per group) was done by 6-week supplementation of rodent chow with 0.2% (w/w) cuprizone (bis-cyclohexanone-oxaldihydrazone; Sigma Aldrich, St. Louis, MO, USA), as described previously (Crocker et al., 2011). The remaining animals (n = 6 per group) were fed with standard rodent chow and served as control groups. The feed was changed 3 times per week and mice were monitored daily for either any abnormal behavior changes or abnormal clinical signs. Weight gain was measured daily during the first week of cuprizone treatment, then every 2–3 days until the end of the experiment. After 6 weeks of cuprizone treatment, animals were euthanized and tissues were processed as described below.

In the second set of experiments, after 2 weeks of cuprizone feeding was performed as described in the first set of experiments, either intravenous transplantation of mouse embryonic stem cells (mES) or DMEM injection was performed in ICR mice (n = 8). Briefly, mice were intravenously injected into the tail vein with either 100 µL of media containing 5 × 10^5 mES cells (n = 5; mES cells-inoculated group), or media alone (n = 3), which served as a sham-inoculated group. Mice from both groups were kept on the cuprizone diet for an additional 2 weeks prior to euthanasia. An additional group of mice (n = 3) were fed with standard rodent chow without addition of cuprizone, and served as the non-lesion control group.

Tissue processing

Mice were deeply anesthetized by an intraperitoneal injection of ketamine (100 mg/kg), and xylazine (15 mg/kg) followed by an intraperitoneal injection of overdose pentobarbitone sodium. Transcardial perfusion was done using 20 mL of sterile PBS followed by 4% paraformaldehyde in PBS, as previously described (Hansmann et al., 2012). Thereafter, a complete necropsy was performed and the observed gross pathological lesions were recorded. For histopathological examination of demyelination and hepatotoxicity, the brains and livers were removed and fixed in 10% neutral buffered formalin overnight. Then they were processed and embedded in paraffin blocks for further histopathological and immunohistochemical evaluation, as described below.

Immunohistochemistry

Immunohistochemistry was performed using the avidin-biotin complex method, as described previously (Hansmann et al., 2012; Pringproa et al., 2015), with minor modification. Briefly, the paraffin-embedded sections were dewaxed, rehydrated and microwave for 30 minutes in citrate buffer (pH...
Sections were incubated for 5 minutes with 3% H$_2$O$_2$ in methanol, and then blocked for 1 hour at room temperature with PBS containing 5% normal goat serum, 0.1% Triton X-100. Thereafter, they were incubated overnight at 4°C with primary antibodies. The primary antibodies used in this study were the mouse monoclonal anti-2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) (1:400; Thermo Scientific, IL, USA), anti-glial fibrillary acidic protein (GFAP) (1:400; Sigma Aldrich, MO, USA), and anti-ionized calcium binding adaptor molecule-1 (Iba-1) (1:200; EMD Millipore, CA, USA). After washing, sections were incubated for 45 minutes at room temperature with biotinylated goat anti-mouse IgG secondary antibody (1:200; Vector Laboratories, CA, USA), followed by peroxidase-coupled avidin-biotin complex (VECTASTAIN Elite ABC kit, Vector Laboratories, CA, USA). Antibody binding was visualized using 3,3-diaminobenzidine-tetrahydrochloride (DAB)-H$_2$O$_2$ for 5 minutes at room temperature followed by counterstaining with Mayer’s hemalum. For each antibody, omission of the primary antibody served as a negative control, and normal brain from untreated groups served as a positive control. Slides were observed and photos were taken under a light microscope. Scoring of immunohistochemically positive cells for each antibody in different anatomical regions was done as described below.

**Myelin staining and demyelination scoring**

Coronal sections of the brains with respect to the anatomical location of the fornix region of the corpus callosum (approximately bregma −0.5 to −0.7 mm) (Paxinos and Franklin, 2001) were 4 μm-thick cut and stained with either Luxol fast blue (LFB), or immunohistochemical labelling with specific antibodies as described above. The regions analyzed were cerebral cortex, deep gray matter, median and lateral parts of corpus callosum (illustrated in Figure 2A). Grading of demyelination was done in CNPase or LFB-stained sections, and scored in the regions of interest between 100 and 0 as previously described (Skripuletz et al., 2008; Grosse-Veldmann et al., 2015). Scoring of demyelination was subjectively done directly by three blinded independent observers using a light microscope (Olympus Corporation, Tokyo, Japan), and was scored as follows: complete demyelination (91–100%) = score 0, moderate demyelination (41–90%) = score 1, mild demyelination (10–40%) = score 2, and normal myelin = score 3.

For quantification of astrogliosis and microgliosis, pictures of selected brain regions were taken using Olympus CX31 microscope (Olympus Corporation, Tokyo, Japan), and Canon EOS 550D (Canon Inc., Tokyo, Japan) digital camera. Then, they were analyzed using the ImageJ (National Institutes of Health, Bethesda, MD). Both GFAP-positive and Iba-1-positive cells were only counted when a cell nucleus was clearly visible, and were given in cells per square millimeter.

**Statistical analysis**

Statistical analyses were accomplished using GraphPad Prism 5 (GraphPad Inc., La Jolla, CA, USA). Either chi-square, or one-way analysis of variance of mean or median followed by Tukey’s post hoc test was performed according to the type of data. Results are shown as the mean ± SE. Statistical significance was designated as $P \leq 0.05$.

**Results**

**Effect of cuprizone on ICR and BALB/c mice**

In the first set of experiments, we aimed to investigate whether ICR mice could be used as an alternative outbred mouse model of demyelination. To determine this, ICR mice were fed with 0.2% cuprizone for 6 consecutive weeks; then body weight and clinical behavior were observed and compared with those of untreated mice. In order to determine the effects of cuprizone-induced demyelination, BALB/c mice were also included in the study. The mean body weight of both ICR and BALB/c mice fed with 0.2% cuprizone was less when compared with the untreated control groups, but the difference of the mean body weight gain was not significant ($P = 0.515$ and $P = 0.303$, respectively) (Figure 2A). Additionally, both ICR and BALB/c mice fed with 0.2% cuprizone showed a mild degree of lethargy compared with the untreated mice (data not shown). The toxic effects of a cuprizone diet on the liver were further analyzed by histopathological observation. Similar to previous reports (Suzuki, 1969; Hiremath et al., 1998), cuprizone-treated ICR and BALB/c mice showed diffuse enlargement of hepatocytes, up to 2–3 times greater than control mouse hepatocytes (Figure 2B). The enlarged hepatocytes were predominantly characterized by increased size of the nucleus and cytoplasm and loose density of the cellular matrix, with occasional multinucleated hepatocytes and degenerated cells (Figure 2B).

To investigate whether cuprizone induces demyelination in ICR and BALB/c mice, different areas of brain sections (Figure 2A), including the cerebral cortex, deep gray matter, and the middle and lateral corpus callosum, were analyzed by either LFB stain or immunohistochemistry for CNPase, and compared with the control groups (Figure 2B–D). The demyelination scores indicated that after 6 weeks of 0.2% cuprizone administration, there was a significant loss of myelin in the corpus callosum ($P < 0.005$ and $P < 0.0001$) and cerebral cortex ($P < 0.005$ and $P < 0.001$) of both ICR and BALB/c mice, respectively, compared with untreated animals (Figure 2B–D). In contrast, the scores of demyelinating lesions in the area of deep gray matter were shown to be not significantly different ($P = 0.055$) between cuprizone-treated and untreated groups of ICR mice (Figure 2B–D). These results indicated that feeding of 0.2% cuprizone induced a region-specific demyelination in ICR mice, but nevertheless could be used as an alternative animal model for demyelinating disease. In addition, cuprizone administration in ICR mice induced a predominant accumulation of astrocytes and microglia in the demyelinated areas, as indicated by positive immunohistochemistry for GFAP and Iba-1, respectively, similarly to that of BALB/c mice (Figure 3A, B).

**Transplantation of mES cells reduced demyelination in ICR mice**

In the second set of experiments, we aimed to study whether myelination in the cuprizone-treated ICR mice could be restored by exogenous cell replacement. To investigate this, mES cells were intravenously inoculated into the tail vein of mice after 2 weeks of cuprizone feeding, followed by an additional 2 weeks of cuprizone exposure. Transplantation of mES cells in the ICR mouse did not cause teratoma formation,
Figure 1 Mean body weight and liver toxicity of ICR and BALB/c mice following cuprizone (Cupri) exposure. (A) Mean body weight and mean body weight gain of ICR outbred and BALB/c inbred mice in Cupri-treated and control (without Cupri treatment) groups. Feeding with 0.2% Cupri for 6 consecutive weeks did not alter the mean body weight gain of ICR and BALB/c mice. (B) Feeding of Cupri in both BALB/c and ICR mice induced liver toxicity as evidenced by diffuse enlargement of hepatocytes, up to 2–3 times greater than control mouse hepatocytes (hematoxylin-eosin stain). The enlarged hepatocytes were predominantly characterized by increased size of the nucleus and cytoplasm, and loose density of the cellular matrix, with occasionally degenerated cells (arrows). Scale bars: 200 µm.

Figure 2 Areas of scoring demyelination, luxol fast blue stain and immunohistochemistry in different brain regions of ICR and BALB/c mice with and without cuprizone (Cupri) exposure. (A) Regions of the mouse brain where tissues were histologically analyzed. (B) Demyelination scores in different brain regions. Higher scores indicate more severe demyelination. (C, D) Representative images of demyelination determined by LFB stain (C) and immunolabelling with CNPase (D). Scoring of demyelination was done in CNPase or LFB-stained sections by three independent observers. (B–D) Feeding of Cupri for 6 weeks induced demyelination in both BALB/c and ICR mice, observed predominantly in the corpus callosum and cerebral cortex. Demyelination in the deep gray matter was observed only in the BALB/c mice, but not in ICR mice. Data in B are shown as the mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 (one-way analysis of variance followed by Tukey's post-hoc test). mCC: Middle corpus callosum; lCC: lateral corpus callosum. Scale bars: 200 µm.
Figure 3 Astrogliosis and microgliosis in ICR and BALB/c mice with and without cuprizone (Cupri) exposure. Numbers of GFAP- (A) and Iba-1-positive (B) cells in the corpus callosum, and representative images of astrogliosis and microgliosis in the Cupri-fed ICR and BALB/c mice. Administration of Cupri significantly increased the number of GFAP- and Iba-1-positive cells (arrows) in the corpus callosum of both ICR and BALB/c mice. Data are shown as the mean ± SE. ***P < 0.001 (one-way analysis of variance followed by Tukey's post-hoc test). lCC: Lateral corpus callosum; GFAP: glial fibrillary acidic protein; Iba-1: ionized calcium-binding adapter molecule 1. Scale bars: 200 µm.

Figure 4 Intravenous transplantation of mouse embryonic stem (mES) cells did alter demyelination scores of cuprizone (Cupri)-fed ICR mice. A Cupri diet was given to the ICR mice 2 weeks prior to intravenous transplantation of mES cells into the tail vein. After 4 weeks of Cupri administration, demyelination score characterized by positive immunoreactivity for CNPase (arrows) in the cerebral cortex and corpus callosum was determined by three independent observers. Transplantation of mES cells significantly improved myelination scores in Cupri-treated ICR mice compared with control (non-lesion) groups. **P < 0.01, ***P < 0.001 (one-way analysis of variance followed byTukey's post-hoc test). lCC: Lateral corpus callosum. Scale bars: 200 µm.
as determined by gross and histopathological evaluation (data not shown). Moreover, transplantation of mES cells in this study exerted a beneficial effect on cuprizone-treated mice, as determined by immunocytochemical labeling with CNPase antibody (Figure 4). Intravenous transplantation of mES cells showed a statistically significant increase in myelination scores for the cerebral cortex ($P = 0.003$) and corpus callosum ($P = 0.002$) of ICR mice compared with those of the sham-inoculated group (Figure 4).

Discussion
The cuprizone mouse model has long been known to be applicable for the study of demyelinating diseases in humans, such as multiple sclerosis (Ludwin, 1978; Kipp et al., 2009; Zendedel et al., 2013). Until recently, cuprizone has been used to induce demyelination in several inbred mouse strains, such as Albino, BALB/c, BSVS, CD1, ICI, and SJL mice (Ludwin, 1978; Skripuletz et al., 2008; Taylor et al., 2009; Skripuletz et al., 2011; Praet et al., 2014). Treatment of inbred mice with cuprizone for 4 consecutive weeks induced prominent demyelination in different brain regions, and the severity of the lesions was shown to be age-, gender-, and strain-dependent (Skripuletz et al., 2008; Taylor et al., 2009). To the best of our knowledge, however, the effects of cuprizone in outbred mouse stocks remain unclear. Outbred mouse stocks offer a diverse genetic background and therefore are expected to have diverse phenotypes, which is closer to the situation in humans (Vaickus et al., 2010). All animal models only partly represent the processes of MS, with each having their own advantages and disadvantages (Skripuletz et al., 2010), and it is not known whether the responses of outbred mice to cuprizone are similar to those of inbred mice; hence, it is worth investigating the effects of cuprizone in outbred mouse stocks.

In the present study, we have shown for the first time that cuprizone-induced demyelination can be observed in ICR outbred mice. To establish a new outbred mouse model of demyelination in this study, we used inbred BALB/c and outbred ICR mice because they are the most widely used strains for various brain behavioral and functional tests (Jung et al., 2014). Moreover, BALB/c mice have been reportedly to develop, despite less demyelinating lesions in the CNS similarly to that C57BL/6 mice (Skripuletz et al., 2008), the most widely used mouse strain in study of cuprizone-induced demyelination (Blakemore, 1973; Matsushima and Morell, 2001; Skripuletz et al., 2010). Our results indicated that feeding ICR outbred mice with a 0.2% cuprizone diet for 6 consecutive weeks induced demyelination in the CNS, and that this model could be used as an alternative mouse strain for the study of demyelination and remyelination. Similar to the effects in BALB/c and C57BL/6 mice, cuprizone-induced demyelination in ICR mice was prominent in the cerebral cortex and corpus callosum (Hiremath et al., 1998; Skripuletz et al., 2008). In contrast to BALB/c mice, however, the results showed that demyelination was not observed in the deep gray matter of ICR mice. Our results further underline the fact that cuprizone-induced demyelination in mice differs depending on the mouse strain (Skripuletz et al., 2008; Taylor et al., 2009).

To investigate whether demyelination in ICR mice caused by cuprizone is ameliorated by exogenous cell replacement, we transplanted mES cells in this newly developed animal model. Since transplantation of embryonic stem cells is known to have potent tumorigenic effects (Arai et al., 2006; Perrone et al., 2012), we investigated the presence of tumor formation in mice by both gross and histopathological examination. In the present study, ICR mice with cuprizone-induced demyelination that were treated by intravenous transplantation of mES cells showed no signs of teratoma formation. Interestingly, transplantation of mES cells potentially attenuated cuprizone-induced demyelination in the present animal model. Furthermore, although mES cells used in the present study were obtained from the B6DZF1 mouse strain, we did not observe the signs of host-anti-graft immunity. Immunogenicity against transplanted ES cells increases upon differentiation stage of ES cells, which requires a certain period after cell transplantation (Swijnenburg et al., 2005).

Although there have been some studies showing that administration of mesenchymal stem cells from various species, including humans, murines and canines, did not improve remyelination scores for cuprizone-induced demyelination (Nessler et al., 2013; Salinas Tejedor et al., 2015), our results were consistent with other previous reports that intravenous transplantation of bone marrow-derived mesenchymal stem cells or human embryonic stem cell-derived neural progenitor cells could enhance remyelination and induce oligo/neuroprotection in mice with cuprizone-induced demyelination (Crocker et al., 2011; El-Akabawy and Rashed, 2015). However, in contrast to the previous studies, we could not demonstrate the presence of mES cells in the remyelinated areas. Thus, the obtained results did not allow us to conclude that it was transplanted cells that migrated and differentiated into CNPase-positive oligodendrocytes. Although transplantation of mES cells ameliorated demyelination scores in the present study, it remains to be determined whether it was the transplanted cells themselves, or trophic effects from the transplanted cells, that enhanced remyelination.

To summarize, the results of these experiments demonstrated that ICR outbred mice could be used as an alternative mouse strain for the study of de- and remyelination. Reduction of demyelination scores following intravenous mES cell injection found in the present study suggested the potential role of exogenous cell transplantation in this newly developed animal model of demyelinating disease.

Acknowledgments: We would like to thank N. Wangnai and W. Chorchakul from the Central Laboratory Unit, Faculty of Veterinary Medicine, Chiang Mai University, Thailand for their technical assistance, J. Sritun and K. Boonsri from the Veterinary Diagnostic Laboratory, Faculty of Veterinary Medicine, Chiang Mai University, for their helpful evaluation of the lesion scores. Author contributions: KP performed the main experiment work and wrote the paper. AS performed the transplantation protocol and wrote the paper. CK and SS performed the animal experiment and special stain. AO provided the mES cells and wrote the paper. All authors approved the final version of the paper. Conflicts of interest: None declared.
Plagiarism check: This paper was screened twice using CrossCheck to verify originality before publication.

Peer review: This paper was double-blinded and stringently reviewed by international expert reviewers.

References

Adamo AM, Paez PM, Escobar Cabrera OE, Wolson M, Franco PG, Pasquini JM, Soto EF (2006) Remyelination after cuprizone-induced demyelination in the rat is stimulated by apotransferrin. Exp Neurol 198:519-529.

Arai Y, Arai H, Asagai A, Yamagata T, Mitani K, Kubota K, Kawamata H, Imai Y (2006) A caspase-3 gene enhance of donor cell origin after allogeneic peripheral blood stem cell transplantation. Am J Transplant 6:3042-3043.

Basoglu H, Boylu NT, Kose H (2013) Cuprizone-induced demyelination in Wistar rats: electrophysiological and histological assessment. Eur Rev Med Pharmacol Sci 17:2711-2717.

Blakemore WF (1973) Demyelination of the superior cerebellar peduncle in the mouse induced by cuprizone. J Neurol Sci 20:63-72.

Cammer W (1999) The neurotoxicant, cuprizone, retards the differentiation of oligodendrocytes in vitro. J Neurosci 16:116-120.

Chia R, Achilli F, Festing MF, Fisher EM (2005) The origins and uses of mouse outbred stocks. Nat Genet 37:1181-1186.

Crooke SG, Moore CS, Frausto RF, Brown GD, Pagarian RR, Whitton JL, Terskikh AV (2011) Intravenous administration of human embryonic stem cell-derived neural precursors attenuates cuprizone-induced central nervous system (CNS) demyelination. Neuroproth Appl Neurobiol 37:643-653.

Dendrou CA, Fugger L, Friesie MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545-558.

Doungpunta J, Santhi A, Sathanawongs A, Jarujinda Y, Oranratnachai S, Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK (2015) Lesion expansion in experimental demyelination animal model of asthma. Comp Med 60:420-426.

Einstein O, Friedman-Levi Y, Grigoriadis N, Ben-Hur T (2009) Transplantation of neural precursors enhance host-brain-derived myelin regeneration. J Neurosci 29:15694-15702.

El-Akabawy G, Rashed LA (2013) Promotion of remyelination by adipose mesenchymal stem cell transplantation in a cuprizone-induced model of depression and anxiety in mice. Biomol Ther (Seoul) 21:453-459.

Kipp M, Clarner T, Dang J, Corpay S, Beyer C (2009) The cuprizone animal model: new insights into an old story. Acta Neuropathol 118:723-736.

Koutsoudaki PN, Hildebrandt H, Gudi V, Skripuletz T, Skuljec J, Stangel M (2010) Remyelination after cuprizone induced demyelination is accelerated in mice deficient in the polysialic acid synthesizing enzyme ShtatA. Neuroscience 171:235-244.

Koutsoudaki PN, Skripuletz T, Gudi V, Moharegh-Khiabani D, Hildebrandt H, Trebst C, Stangel M (2009) Demyelination of the hippocampus is prominent in the cuprizone model. Neurosci Lett 451:83-88.

Kudwin SK (1978) Central nervous system demyelination and remyelination in the mouse: an ultrastructural study of cuprizone toxicity. Lab Invest 39:597-612.

Matsushima GK, Morell P (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 11:107-116.

Nessler J, Benardais K, Gudi V, Hoffmann A, Salinas Tejedor L, Jansen S, Prajeeth CK, Baumgartner W, Kavelaars A, Heijnen CJ, van Peppelen C, Hausmann F, Skripuletz T, Stangel M (2013) Effects of murine and human bone marrow-derived mesenchymal stem cells on cuprizone induced demyelination. PLoS One 8:e69795.

Paxinos G, Franklin RJ (2001) The Mouse Brain in Stereotaxic Coordinates. San Diego: Academic Press.

Perrone-Franceschi I, Barberi W, Valle V, Torelli GF, Natalino F, Mercant C, Foa R, Iori AP (2012) First case of ovarian Sertoli-Leydig cell tumor after an allogeneic hematopoietic stem cell transplantation for Hodgkin's disease. Leuk Res 36:e185-186.

Pretz J, Guglielmetti C, Berneman Z, Van der Linden A, Ponsaerts P (2014) Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. Neurosci Biobehav Rev 47:485-505.

Pringproa K, Pringproa K, et al. / Neural Regeneration Research. 2016;11(10):1603-1609.

Rodriguez M (2007) Effectors of demyelination and remyelination in the CNS: implications for multiple sclerosis. Brain Pathol 17:219-229.

Suzuki K, Hirose Y, Hirata K, Tsuchida S, Nishibata T, Hasegawa M, Matsumura T, Takecugu H, Arai Y, Arai H, Aoyagi A, Yama gata T, Mitani K, Kubota K, Kawamata H, Imai Y (2006) A caspase-3 gene enhance of donor cell origin after allogeneic peripheral blood stem cell transplantation. Am J Transplant 6:3042-3043.