

Scientific Report

Administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the intracranial hemorrhage in two dogs: a case report

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Summary

Two dogs with generalized seizures were evaluated. The dogs were diagnosed with traumatic intracranial hemorrhages based on the history, neurological examinations, and magnetic resonance imaging (MRI) of the brain. Treatment was started with oxygen, prednisolone and anticonvulsant agents. No further seizure activity was observed after treatment in both dogs, however cushing reflex was detected in case 1 and a left-sided hemi-paresis was detected in case 2. Further supportive treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF) was attempted. No abnormal signs were noted in either of the dogs and no recurrence was noted 16 and 14 months later, in case 1 and 2, respectively. These cases indicate that a combination of rhG-CSF treatment with previous therapy could be used in dogs with traumatic brain injury.

Key words: Granulocyte colony-stimulating factor, Intracranial hemorrhage, Magnetic resonance imaging, Traumatic brain injury

Introduction

Traumatic brain injury (TBI) can occur mostly from an external force such as in an automobile trauma, falls and rarely gun-shots (Kitagawa *et al.*, 2005). Traumatic brain injury may or may not be related to intracerebral hemorrhage, subarachnoid hemorrhage, and intraventricular hemorrhage (Sande and West, 2010). Intracranial hemorrhage causes high mortality and morbidity, neurologic damage and remains in approximately 10% of all strokes in humans (Manno *et al.*, 2005). Direct external forces refer to primary lesions, which have already occurred when the patient is presented (Sosin *et al.*, 1996). Thus the prevention of further neurologic secondary damage from hemorrhage, ischemia, edema formation, seizure and increased intracranial pressure (ICP) is important (Fishman, 1975; Shapiro, 1975; Bouma *et al.*, 1992).

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) enhances mobilization of hematopoietic stem cells, so it has been used for the treatment of neutropenia (Dale *et al.*, 2003). Recently, several studies demonstrated neuroprotective effects of rhG-CSF by neurogenesis and angiogenesis (Lu and Xiao, 2006; Sehara *et al.*, 2007; Diederich *et al.*, 2009). In these previous studies we then attempted to examine the effect of rhG-CSF on the progression of TBI. This case report represents the serial magnetic resonance imaging of TBI induced intracranial hemorrhage and clinical outcomes with rhG-CSF administration in two dogs.

Case presentation and diagnosis

A 4-year-old, 2.2-kg, intact male Maltese dog (case 1) and a 9-year-old, 2.4-kg, intact female Yorkshire terrier dog (case 2) were presented with progressive seizure after head trauma. Both dogs showed a tonic-clonic seizure immediately after the injury. At presentation, facial myoclonus and tonic-clonic seizure was detected in case 1 and case 2, respectively. Physical examination revealed a depressed mental status in both dogs. Systolic blood pressure (Cardell Model 9401, Sharn Veterinary Inc., FL, USA) was elevated in case 1 (152 mmHg) and normal in case 2 (130 mmHg). Neurological examination revealed a decreased menace response in the right eye (both dogs) and decreased postural reactions on the left side limbs in case 2. A complete blood count (CBC) was normal in case 1 and leukocytosis with stress leukogram was detected in case 2. A serum chemistry profile of case 1 showed elevated alanine aminotransferase (ALT) (498 U/L, reference range; 19-70U/L), aspartate transferase (AST) (59 U/L, reference range; 15-43 U/L), lactic dehydrogenase (150 IU/L, reference range 0 to 130 IU/L), creatine kinase (338 IU/L, reference range 46 to 320 IU/L) and blood glucose (172 mg/dl, reference range 70 to 118 mg/dl). In case 2, elevated ALT (740 U/L), AST (94 U/L), and CK (989 U/L) was detected. During examination, case 1 experienced tonic-clonic seizures and PLR had disappeared with miosis. The dog was treated with 4 mg/kg phenobarbital sodium (Luminal; Dai Han Pharm, Seoul, Korea) intravenously. Based on the clinical signs,

a physical and neurological examination, as well as the apparent history, an intracranial lesion was suspected.

A brain MRI scan using a 0.2 T (E-scan®; ESAOTE, Genova, Italy) was performed 6 days (case 1) and 1 day (case 2) after the injury. In case number 1, transverse T2- and T1-weighted images (WI) revealed hyperintense lesions in the right amygdaloid body in the piriform lobe (Figs. 1A and B). Dorsal T2-WI also showed diffuse hyperintensities in those areas (Fig. 1C). These lesions were not enhanced on T1-WI after intravenous administration of gadolinium (Omniscan; Amersham Health, USA; 0.1 mmol/kg, IV). Follow-up MRI scans taken 62 days after admission (Figs. 1D-F). In case number 2, a transverse T2-WI lesion and a transverse T1-WI hypointense lesion were revealed at the left occipital lobe (Figs. 2A and B). Dorsal T2-WI also showed hyperintensities (Fig. 2C). Dilation of the bilateral lateral ventricles was also noted (Fig. 2D). In both dogs, RT-PCR for canine distemper virus was negative in the serum and CSF. Case 1 was diagnosed with TBI and case 2 was diagnosed with hydrocephalus and TBI.

Treatment was initiated with continuous oxygen, isotonic intravenous (IV) fluids, a combination of 1 mg/kg prednisolone (Solondo; Yuhan Medica, Korea) twice daily per oral and 3 mg/kg phenobarbital (Phenobarbital; Myung-In Pharm, Korea) twice a day per oral followed by a 1 g/kg constant rate infusion of mannitol (d-mannitol 20%; Dai Han Pharm, Korea) over 30 min. The steroids were tapered rapidly over 5 days. In case 2, 0.5 mg/kg furosemide (Lasix; Handok, Korea) twice daily, and 0.7 mg/kg omeprazole (Omeprazole; Sinil Pharm, Korea) once daily per oral was added to the regimen. For neuroprotection, a subcutaneous injection of rhG-CSF (10 µg/kg, Leukokain; CJ Pharm, Korea) for 3 days was decided in both dogs. During rhG-CSF administration, no abnormal signs were detected. CBC and flow cytometric analysis by the dual-platform

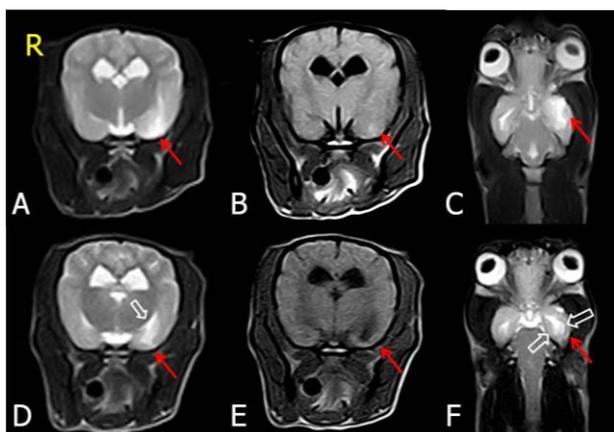


Fig. 1: MR images of case 1. Hyperintense lesions (red arrow) of transverse T2- (A) and T1-WI (B) on the left amygdaloid body in the piriform lobe were demonstrated. (C) T2-weighted coronal image of the same lesions. A transverse T2- (D) and T1-WI (E), and T2-weighted coronal (F) image of the same dog 2 months after injury. Hyperintense lesions (red arrow) were demarcated by a sharply delineated hypointense margin (open arrow) on T2-WI (D) and a hypointense signal on the lesions (E) which was previously a hyperintense signal on the T1-WI

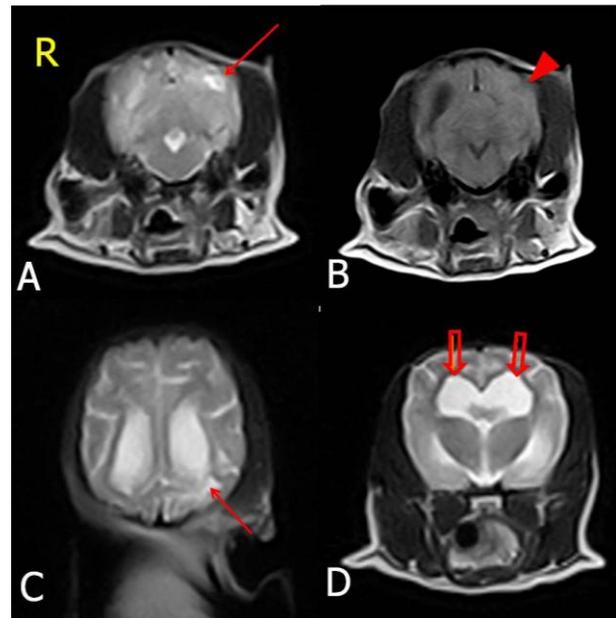


Fig. 2: MR images of case 2. Hyperintense lesions (red arrow) of transverse T2-WI (A) and hypointense lesions (arrow head) of transverse T1-WI (B) on the left occipital lobe were demonstrated. (C) Coronal T2-WI of the same lesions also showed hyperintensities (red arrow) and dilation of the bilateral lateral ventricles was also noted. (D) Transverse T2-WI of dilation of the bilateral lateral ventricles (open arrow) was demonstrated at the level of the thalamus

method were performed before and after rhG-CSF treatment to evaluate the mobilization of hematopoietic stem and progenitor cells (HSPCs) from the bone marrow into the peripheral blood (Fig. 3). Seven days after the initial treatment, there was no other seizure episode and behavior changes were improved in case 1. The systemic blood pressure (Cardell Model 9401, Sharn Veterinary Inc., FL, USA) and the heart rates were monitored (Fig. 4). In case 2, seizure was only detected 2 more times after the treatment; however a left-sided hemi-paresis was detected after 7 days and it resolved slowly. The clinical signs were resolved completely two weeks after presentation and no recurrence was detected over 14 months (case 2). At 62 days after admission, intracranial lesions were re-examined through MRI in case 1. The lesions were decreased and demarcated by a sharply delineated hypointense margin (Figs. 1D-F). The phenobarbital started to slowly taper off and no further seizures were detected over 16 months.

Discussion

Intracranial hemorrhage from any reason causes cerebral dysfunction due to the local mass effect, which induces intracranial volume expansion and edema formation (Xi *et al.*, 2001). Cerebral edema persists for 3 to 7 days and results in secondary ischemia and/or elevated intracranial pressure (Xi *et al.*, 2002; Bouzat *et al.*, 2013). In this report, both dogs had TBI and secondary neurologic problems such as seizure, behavior change, and ataxia. The case 1 dog only had TBI and no

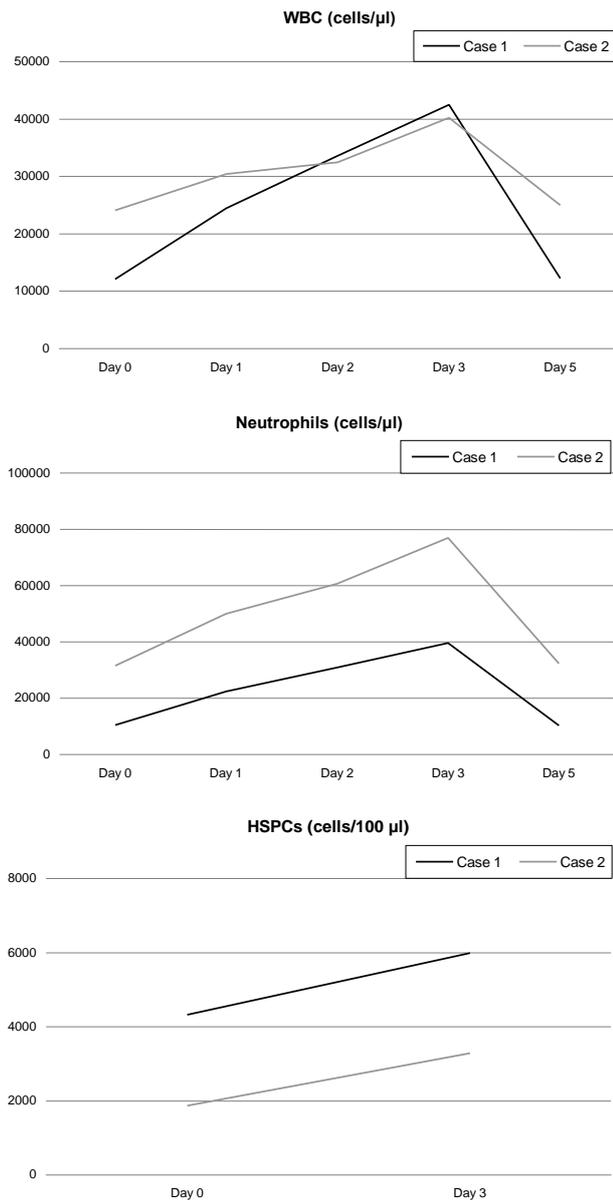


Fig. 3: Total leukocyte counts, neutrophil counts and HSPCs before and after rhG-CSF treatment in case 1 and case 2. After the first dose, the leukocyte and neutrophils counts increased rapidly and decreased after discontinued use of the rhG-CSF. HSPCs were increased during the rhG-CSF treatment periods, (day 0; before rhG-CSF treatment)

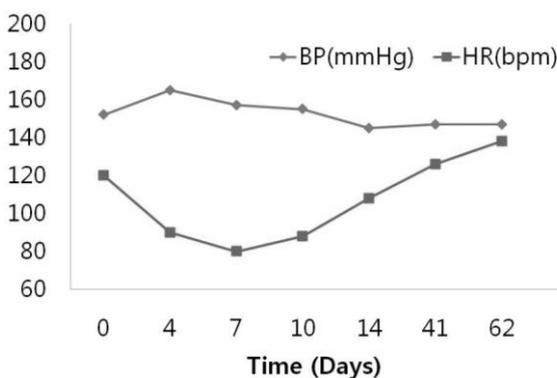


Fig. 4: Blood pressure and heart rate change following head trauma in case 1

underlying diseases were revealed, however case 2 was diagnosed as hydrocephalus and TBI based on the history and MRI findings.

An MRI scan was performed 6 days after the trauma in case 1 and 1 day after the trauma in case 2. In case 1, both T1-WI and T2-WI showed elevated signals, whereas case 2 showed hypointense T1-WI and hyperintense T2-WI. According to the previous reports (Huisman, 2005; Jadhav *et al.*, 2008), the brain MRI of case 1 was consistent with early subacute (period of time from 3 to 7 days after onset) images of hemorrhage and case 2 was consistent with acute (period of time from 24 to 72 h after onset) images of hemorrhage. Moreover, a serial MRI scan was achieved in case 1 and chronic changes of the hemorrhagic lesions were also detected.

The treatment goal for the intracranial hemorrhage is to maintain cerebral perfusion and subsequent tissue oxygenation, as well as the management of secondary neurologic sequelae such as seizures, and the treatment of any underlying diseases (Manno *et al.*, 2005). Recent studies revealed that TBI could induce neurodegeneration and the use of neuroprotective agents was helpful for the suppression of the progression of disease (Lu and Xiao, 2006; Sehara *et al.*, 2007; Diederich *et al.*, 2009). In these 2 cases, we used rhG-CSF for potential neuroprotective and neurogenesis effects. According to recent reports on animal models of ischemic brain injury (Six *et al.*, 2003; Gibson *et al.*, 2005; Khatibi *et al.*, 2011), several mechanisms including anti-inflammatory effects, inhibition of apoptosis and stimulation of endogenous stem cell proliferation could mediate the neuroprotective effects of rhG-CSF. The direct effects of rhG-CSF to the brain could not be evaluated in these 2 cases because the dogs were still alive; however, the effects of rhG-CSF were indirectly evaluated through improved clinical signs of the dogs, elevated neutrophil count and increased HSPCs (England *et al.*, 2012). Moreover, a subsequent MRI scan was performed in case 1. Further trials for the treatment of TBI dogs with rhG-CSF are required to establish the potential therapeutic effects of rhG-CSF.

In conclusion, these cases demonstrate that the combination of rhG-CSF administration with previous TBI therapy including anti-inflammatory, anti-oxidant therapy, and decrease of intracranial edema could be potentially used in dogs with TBI due to the further neuroprotective effects of rhG-CSF through the inhibition of apoptosis and stimulation of endogenous stem cell proliferation. However, further clinical studies are required, since the mechanisms of rhG-CSF as neuroprotective agents are still not fully understood.

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