A Case of Acquired Aplastic Anemia with Repeated Cerebral Infarctions at The Beginning of Immunosuppressive Therapy

Rikio SUZUKI[*1, *2], Ako KIKUCHI[*1, *2], Daisuke OHGIYA[*2], Hiromichi MURAYAMA[*2], Masako TOYOSAKI[*2], Takahiro SUYAMA[*1], Shigeki WATANABE[*1], Yoshiaki OGAWA[*2], Hiroshi KAWADA[*2] and Kiyoshi ANDO[*2]

[*1]*Division of Hematology, Ebina General Hospital*
[*2]*Department of Hematology / Oncology, Tokai University School of Medicine*

(Received April 27, 2009; Accepted June 1, 2009)

Abstract: Acquired aplastic anemia is a rare hematopoietic stem-cell disorder that results in pancytopenia and hypocellular bone marrow. The pathophysiology is immune mediated in most cases, with activated type 1 cytotoxic T cells implicated. Acquired aplastic anemia can now be cured or ameliorated by stem-cell transplantation or immunosuppressive drug therapy such as antithymocyte globulin or cyclosporine. We present a rare case report of a 68-year old patient with acquired severe aplastic anemia with repeated cerebral infarctions at the beginning of immunosuppressive therapy. He started immunosuppressive drug therapy with antithymocyte globulin and cyclosporine. During follow-up, magnetic resonance imaging revealed high signals at right thalamus and right pons by diffusion-weighted image. He was diagnosed with repeated cerebral infarctions of right thalamus and right pons. We successfully managed cerebral infarctions by frequent transfusions, edaravone administration, keeping the trough of serum cyclosporine (CsA) concentration around lower limit. This is the first report of successful management of acquired aplastic anemia with repeated cerebral infarctions.

Keywords: Acquired aplastic anemia, Cerebral infarction, Klebsiella oxytoca infection, Cyclosporine, Edaravone

INTRODUCTION

Acquired aplastic anemia (AA) is characterized by peripheral blood cytopenia and reduced marrow cellularity [1]. Recent advances revealed, however, that the most cases, of AA is an immune-mediated disease. It is suggested that an inciting event, such as a virus or medical drug, provokes an aberrant immune response, triggering oligoclonal expansion of cytotoxic T cells against hematopoietic stem cells [2]. In some cases, several somatic mutations such as *TERC*, *TERT* and *Shwachman-Bodian-Diamond syndrome* (*SBDS*) gene mutations have been reported to be associated with development of acquired AA [3, 4].

Definitive therapies for severe AA are immunosuppression or stem cell transplantation. Combination of antithymocyte globulin (ATG) with cyclosporine (CsA) is the corner stone of the immunosuppressive therapy, and yields strong synergy, and lead to the improvement in blood counts [1, 5]. Outcomes of immunosuppressive therapy are related to patient age: 5-year survival of more than 90% of children has been reported in recent several trials, compared with about 50% survival for adults older than 60 years in some trial [2]. Allogeneic transplant from a matched sibling donor cures the great majority of patients [6]. Recent results with alternative sources of stem cells and a variety of conditioning regimens to achieve their engraftment have been promising.

There are some reports of aplastic anemia with infarction. One is about a case of cerebral hemorrhagic infarction associated with anabolic steroid therapy for hypoplastic anemia [7]. Another is about two cases of acute myocardial infarction associated with aplastic anemia during treatment with anabolic steroids [8]. Also, clinical use of CsA was suggested to be associated with an increased risk of thromboembolic complications [9]. However, a case of acquired aplastic anemia with repeated cerebral infarctions has never been found before. Here, we report a case of acquired aplastic anemia with repeated cerebral infarctions at the beginning of immunosuppressive therapy, which succeeded in the management of both acquired AA and cerebral infarctions.

CASE REPORT

A 68-year-old male patient was admitted to our hospital because of pancytopenia. Laboratory data on admission were as follows: red blood cells (RBC) 2180 $\times 10^9$/L; white blood cells (WBC) 2.33 $\times 10^9$/L with 23% neutrophils, 62% lymphocytes, 5% monocytes, 10% eosinophils and no myeloblasts; hemoglobin (Hb) 8.1 g/dL; platelets (Plt) 1.1 $\times 10^9$/L; Ret 1.5 $\times 10^9$/L (Table 1). Bone marrow biopsy showed a markedly hypocellular bone marrow without apparent dysplasia. By cytogenetic analysis, 18 of 20 metaphases examined

Rikio SUZUKI, Division of Hematology, Ebina General Hospital, 1320 Kawaraguchi, Ebina, Kanagawa, Japan. Tel: +81-462-33-1311 Fax: +81-455-31-0169
E-mail: rickey-s@is.icc.u-tokai.ac.jp

Table 1 Laboratory data at onset of AA

WBC	2,330 /μL	Fe	123 μg/dL
RBC	218×10^4/μL	TIBC	201 μg/dL
Hb	8.1 g/dL	UIBC	78 μg/dL
Ht	22.5%	Ferritin	235 ng/mL
MCV	103.0 fL		
MCH	37.0 pg	PT	10.3 sec
MCHC	35.9%	PT%	130 %
Plt	1.1×10^4/μL	APTT	29 sec
Reticulocyte	1.5×10^4/μL	FDP	6.5 μg/mL
		Fibrinogen	329 mg/dL
Total protein	6.9 g/dL		
Albumin	3.5 g/dL	HBs Ag	(-)
AST	40 IU/L	HCV Ab	(-)
ALT	45 IU/L		
LDH	183 IU/L	Vitamin B12	194 pg/mL
ALP	211 IU/L	Folic acid	6.4 ng/mL
BUN	22.6 mg/dL	*H.pylori* Ab	(-)
Cre	0.88 mg/dL		
Na	141 mEq/L	Total cholesterol	200 mg/dL
K	4.1 mEq/L	Triglyceride	102 mg/dL
Cl	108 mEq/L	HDL cholesterol	52 mg/dL
Total bilirubin	0.3 mg/dL	LDL cholesterol	127 mg/dL
Direct bilirubin	0.1 mg/dL	Glucose	151 mg/dL
CRP	<0.09 mg/dL	HbA1C	5.6%

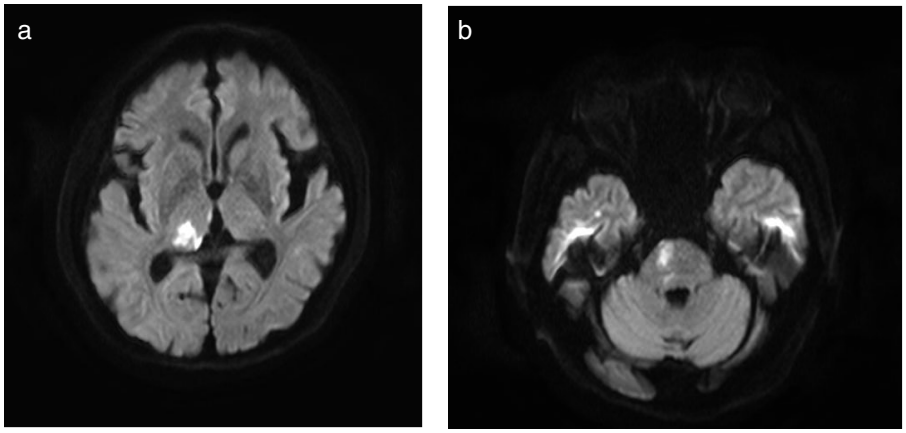

Fig. 1 Magnetic resonance imaging of the patient's brain by diffusion-weighted image (DWI) on day 12 (a) and 13 (b).

were 45, X, -Y and 2 of 20 metaphases examined were 46, XY. Flow cytometric analysis showed that the red blood cells and granulocytes were 100% positive for CD55 and CD59, and there were no paroxysmal nocturnal hemoglobinemia (PNH) clones. He had no symptom of hypertension on admission. Eventually, he was diagnosed as acquired severe aplastic anemia. Immunosuppressive therapy was started with ATG (15 mg/kg, 900 mg/day, day 1−5), CsA (5 mg/kg, 300 mg/day), methylprednisolone (2 mg/kg, 120 mg/day, day 1−5) and prednisone (1 mg/kg, 60 mg/day, day 6−14, 0.5 mg/kg, 30 mg/day, day 15−21, and 0.2 mg/kg, 12 mg/day, day 22−28). He presented with high fever of 40.0˚C, due to an allergic reaction to ATG. Transfusions were properly performed toward anemia and thrombocytopenia during immunosuppressive therapy. From day 7 after the therapy, left anesthesia had been appeared. We suspected an intracranial hemorrhage (ICH) or a subarachnoid hemorrhage and computed tomography (CT) scans were performed. Since they demonstrated only low density area in left basal ganglia maybe due to old infarctions and obvious signs of hemorrhages were not detected, we kept careful observation of the clinical course. However, left anesthesia had been maintained, and magnetic resonance imaging (MRI) was performed on day 15, which demonstrated high signals in right thalamus by diffusion-weighted image (DWI), T2-weighted image and FLAIR (Fig. 1). We diagnosed as acute infarction of right thalamus. There was no significant formation of stenosis, obstruction, or aneurysm in the main arteries by magnetic resonance angiography (MRA) (Fig. 2). On the other hand, he had high fever around 39 ˚C on day 13, and Klebsiella oxytoca was

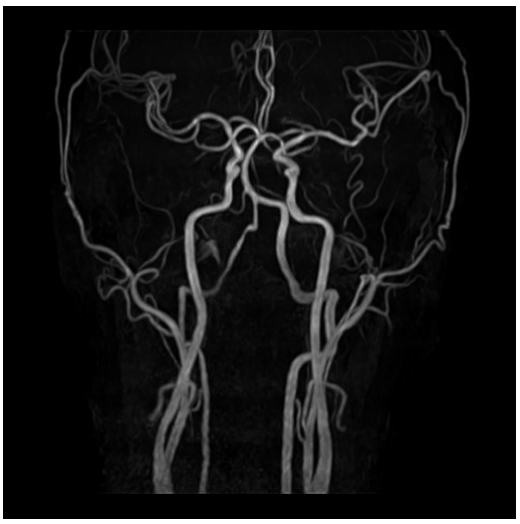

Fig. 2 Magnetic resonance angiography (MRA) on day 13.

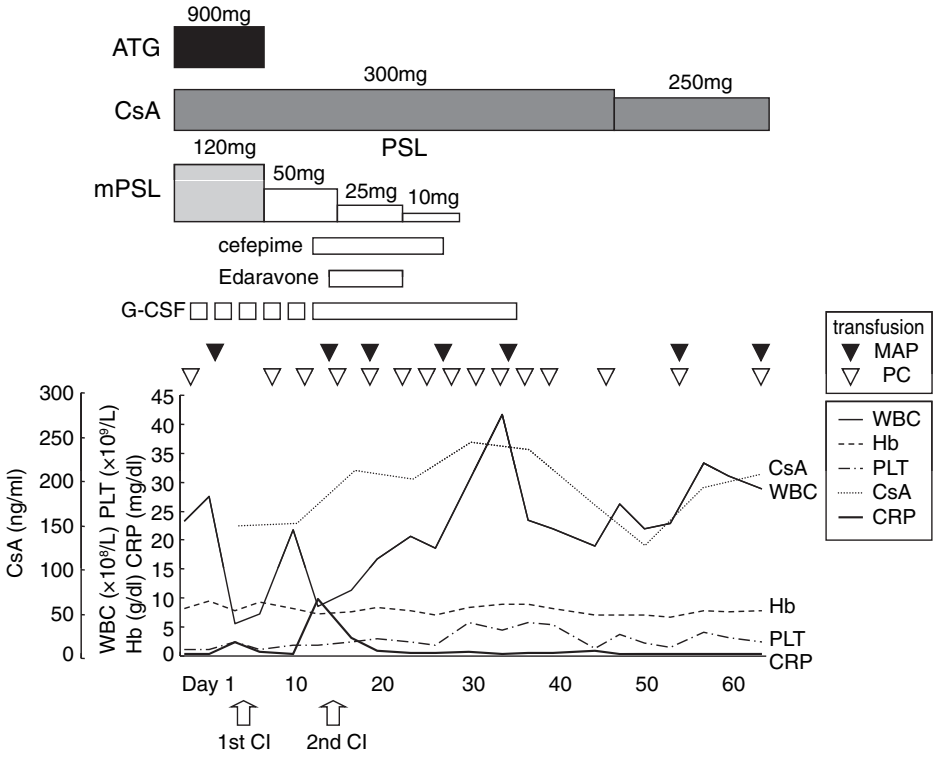

Fig. 3 Serial clinical events and follow-up of the case. Black and white triangles indicate red blood cell and platelet concentrate infusion, respectively. WBC white blood cell, Hb hemoglobin, PLT platelet, CsA cyclosporine A, MAP mannitol adenine phosphate, PC platelet concentrate, CI cerebral infarction, PSL prednisolon, mPSL methyl prednisolon, ATG antithymocyte globulin.

detected in the blood culture on day 13. On day 16, left hemiparesis was newly appeared. Another MRI on day 16 demonstrated the new high signal lesion in right pons by DWI (Fig. 1). So, he was diagnosed with acute infarctions of right pons as a new second lesion. Because the feeding arteries of these infarction lesions are different from each other, we diagnosed as repeated cerebral infarctions of right thalamus and right pons. With regard to acute infarction of right pons, because it was found within 48 hours from sideration, free radical scavenger Edaravone was available, and actually administered every day two weeks. To prevent hemorrhagic infarction, platelet transfusions were repeatedly performed adequately. Toward sepsis due to Klebsiella oxytoca infection, daily subcutaneous injection of granulocyte colony stimmulating factor (G-CSF) and daily intravenous infusion of cefepime (CFPM) had been administered from day 13. He had already been afebrile on day 16, and infectious signs were improved soon. Because it was possible that CsA would stimulate infarction lesions and induce CsA neurotoxicity, the trough of serum CsA concentration had been

controlled around lower limit. Thereafter, the progression of left anesthesia and hemiparesis were not found. After rehabilitation and hematologic recovery, he was discharged on 63 days after admission.

DISCUSSION

We experienced a very rare case of acquired severe aplastic anemia with repeated cerebral infarctions at the beginning of immunosuppressive therapy. A case of acquired aplastic anemia with repeated cerebral infarctions has never been found before. A cerebral infarction is the rapidly developing loss of brain functions due to a disturbance in the blood vessels supplying to the brain [10]. Since the size of infarct in this patient was over 1.5 cm on MRI, diagnosis of subtype of ischemic stroke is considered to be atherosclerosis, according to TOAST classification [11]. Shiozawa *et al.* [7] reported a case of cerebral hemorrhagic infarction associated with anabolic steroid therapy for hypoplastic anemia, and suggested the relationship between cerebral infarction and anabolic steroid therapy. However, he was never diagnosed as a hemorrhagic infarction, and we didn't use anabolic steroid therapy. There are some reports about paroxysmal nocturnal haemoglobinuria (PNH) with thrombosis [12, 13]. Paroxysmal nocturnal hemoglobinuria is a rare, acquired stem cell disorder, with its primary clinical manifestations being hemolytic anemia, marrow failure and thrombophilia. Chronic hemolysis, failures of the fibrinolytic system, increased leukocyte-derived tissue factor levels in plasma, procoagulant microparticles generated through complement-mediated damage of platelets and venous endothelium are related to the acquired hypercoagulable state and thrombosis [14]. In part, it is well-known that AA and PNH can transit to each other [15]. There were no PNH clones in this case, and it was hard to understand that repeated infarctions were associated with PNH. In this case, there would be three possible factors leading to the repeated cerebral infarctions. Firstly, because sepsis is a main cause of the genesis of microparticles, it is suggested that sepsis due to Klebsiella oxytoca contributed to the increased level of microparticles, and caused the hypercoagulable state and thrombosis similar to disseminated intravascular coagulation [16]. Secondly, it is suggested that clinical use of CsA was associated with an increased risk of thromboembolic complications. It is indicated that thrombogenic properties of CsA may result from the alteration of lipid organization in platelet plasma membrane, leading to externalization of phosphatidylserine (PS) and accelerated thrombine generation [9]. So, In this case, clinical use of CsA might invite the repeated cerebral infarctions. Thirdly, it is suggested that hemodynamic factor such as septic shock or septic emboli due to Klebsiella oxytoca infection contributed to both infarctions. In this case, there were no any symptoms of infectious endocarditis which most frequently cause septic emboli [17]. In addition, we could not find any possible focuses of Klebsiella oxytoca. But, because Klebsiella oxytoca usually exist in the digestive tract, it is suggested that this case originated from the digestive tract.

We successfully managed the severe conditions consisted of acute cerebral infarction and pancytopenia by using free radical scavenger edaravone. If the patient does not suffer from acquired aplastic anemia, antiplatelet agent should be administered as the medication for stroke. However, in this case, we did not use anti-platelet agent to avoid hemorrhagic complications which can't be predicted. Edaravone is widely used clinically in Japan for acute cerebral injury, especially cerebral infarction [18]. It is strongly suggested that reactive oxygen species (ROS) are generated and play a harmful role during the acute and late stages of cerebral ischemia. Edaravone scavenges ROS and inhibits proinflammatory responses after brain ischemia in animals and humans [18]. In particular, post ischemic inflammation, leading to brain edema and infarction due to neuronal damage and endothelial cell death, can be ameliorated by edaravone [19]. In addition, recent studies indicate that ROS are involved in persistent pain, including neuropathic and inflammatory pain. So, it is suggested that edaravone is effective on neuropathic pain [20]. Since we could not administer antiplatelet or anticoagulant therapy due to thrombocytopenia, and because he suffered from left anesthesia, it seems that edaravone had been a best choice in this case.

Also, we successfully escaped from CsA neurotoxicity. CsA administration results in a specific inhibition of interleukin production in the T cell, and leads to a strong immunosuppression [21]. Neurotoxicity is one of the most significant clinical side effects of CsA. The clinical symptoms of CsA-mediated neurotoxity consist of depressed responsiveness, hallucinations, delusions, seizures, cortical blindness, and stroke-like episodes that resemble clinical symptoms of mitochondrial encephalopathy. This toxicity might arise from the intersection of CsA with mitochondrial energy metabolism [21]. It has been reported that the incidence of neurotoxicity was positively correlated with high CsA trough levels [22]. So, in this case, because it was possible that repeated CsA might stimulate infarction areas and cause CsA-mediated neurotoxity, the trough of serum CsA concentration had been controlled around lower limit. After all, we successfully escaped from CsA neurotoxicity. If blood cell count could permit lower limit serum concentration trough level of CsA, it would be a best way to escape from CsA neurotoxicity.

REFERENCES

1) Bacigalupo A. Aplastic anemia: pathogenesis and treatment. Hematology Am Soc Hematol Educ Program. 2007; 23–8.
2) Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006(8); 108: 2509–19. doi: 10.1182/blood-2006-03-010777.
3) Calado RT, Young NS. Telomere maintenance and human bone marrow failure. Blood. 2008; 111(9): 4446–55. doi: 10.1182/blood-2007-08-019729.
4) Calado RT, Graf SA, Wilkerson KL, Kajigaya S, Ancliff PJ, Dror Y, *et al*. Mutations in the SBDS gene in acquired aplastic anemia. Blood. 2007; 110(4): 1141–6. doi: 10.1182/blood-2007-03-080044.
5) Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. Semin Hematol. 2000; 37(1): 56–68.
6) Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. Semin Hematol. 2000; 37(1): 30–42.
7) Shiozawa Z, Tsunoda S, Noda A, Saito M, Yamada H. Cerebral hemorrhagic infarction associated with anabolic steroid therapy

for hypoplastic anemia. Angiology. 1986; 37(10): 725–30.
8) Toyama M, Watanabe S, Kobayashi T, Iida K, Koseki S, Yamaguchi I, *et al*. Two cases of acute myocardial infarction associated with aplastic anemia during treatment with anabolic steroids. Jpn Heart J. 1994; 35(3): 369–73.
9) Tomasiak M, Rusak T, Gacko M, Stelmach H. Cyclosporine enhances platelet procoagulant activity. Nephrol Dial Transplant. 2007; 22(6): 1750–6.
10) Warlow C, Sudlow C, Dennis M, Wardlaw J, Sandercock P. Stroke. Lancet. 2003; 362(9391): 1211–24. doi: 10.1016/S0140-6736(03) 14544–8.
11) Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, *et al*. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993; 24(1): 35–41.
12) Audebert HJ, Planck J, Eisenburg M, Schrezenmeier H, Haberl RL. Cerebral ischemic infarction in paroxysmal nocturnal hemoglobinuria report of 2 cases and updated review of 7 previously published patients. J Neurol. 2005; 252(11): 1379–86.
13) Hassan KM, Varadarajulu R, Sharma SK, Motwani H, Mishra DK, Dhall A, *et al*. Cerebral venous thrombosis in a patient of paroxysmal nocturnal haemoglobinuria following aplastic anaemia. J Assoc Physicians India. 2001; 49: 753–5.
14) Ziakas PD, Poulou LS, Pomoni A. Thrombosis in paroxysmal nocturnal hemoglobinuria at a glance: a clinical review. Curr Vasc Pharmacol. 2008; 6(4): 347–53.
15) Brodsky RA. Paroxysmal nocturnal hemoglobinuria: stem cells and clonality. Hematology Am Soc Hematol Educ Program. 2008; 2008: 111–5.
16) Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Rev. 2007; 21(3): 157–71.
17) Ruttmann E, Willeit J, Ulmer H, Chevtchik O, Höfer D, Poewe W, *et al*. Neurological outcome of septic cardioembolic stroke after infective endocarditis. Stroke. 2006; 37(8): 2094–9. doi: 10.1161/01. STR. 0000229894.28591.3f.
18) Watanabe T, Tahara M, Todo S. The novel antioxidant edaravone: from bench to bedside. Cardiovasc Ther. 2008; 26(2): 101–14.
19) Yoshida H, Yanai H, Namiki Y, Fukatsu-Sasaki K, Furutani N, Tada N. Neuroprotective effects of edaravone: a novel free radical scavenger in cerebrovascular injury. CNS Drug Rev. 2006; 12(1): 9–20. doi: 10.1111/j.1527-3458.2006.00009.x.
20) Mao YF, Yan N, Xu H, Sun JH, Xiong YC, Deng XM. Edaravone, a free radical scavenger, is effective on neuropathic pain in rats. Brain Res. 2009; 1248: 68–75. doi: 10.1016/j.brainres. 2008. 10. 073.
21) Serkova NJ, Christians U, Benet LZ. Biochemical mechanisms of cyclosporine neurotoxicity. Mol Interv. 2004; 4(2): 97–107.
22) Magnasco A, Rossi A, Catarsi P, Gusmano R, Ginevri F, Perfumo F, *et al*. Cyclosporin and organ specific toxicity: clinical aspects, pharmacogenetics and perspectives. Curr Clin Pharmacol. 2008; 3(3): 166–73.