

Effects of granulocyte colony-stimulating factor treatment in children and patients with cerebral palsy: a preliminary report

Paszko-Patej G.^{A-F}, Kułak W.^{A-F*}, Okurowska-Zawada B.^{B,D,F}, Sienkiewicz D.^{B,D,F}, Wojtkowski J.^{B,D,F}, Sochoń K.^{B,D,F}, Kalinowska A.^{B,D,F}, Okulczyk K.^{B,D,F}, Topór E.^{B,D,F}, Mirska A.^{B,D,F}

Department of Pediatric Rehabilitation and Center of Early Support for Handicapped Children "Give a Chance," Medical University of Białystok, Białystok, Poland

A - Conception and study design; **B** - Collection of data; **C** - Data analysis; **D** - Writing the paper; **E** - Review article; **F** - Approval of the final version of the article; **G** - Other (please specify)

ABSTRACT

Introduction: Recent reports have revealed that neuroinflammation and apoptosis in brains affected by cerebral palsy could be therapeutic targets. Granulocyte colony-stimulating factor (G-CSF) exerts anti-inflammatory and antiapoptosis effects and stimulates the proliferation of neural stem and progenitor cells in the brain.

Purpose: To assess the efficacy and safety of G-CSF treatment in children and adolescents with CP.

Materials and methods: Six patients with spastic tetraplegia CP aged 3-15 years were enrolled in this study. Five patients had GMFCS (Gross Motor Function Classification System) level at V, three children had epilepsy, and three had severe mental retardation. We used the gross motor function measure-66 (GMFM-66) to assess motor function.

GCSF (5µg/kg/body/day) was administered subcutaneously for five consecutive days during the four months. The parents also evaluated the physical and mental development of their children.

Results: We observed improvement in motor function in patients with CP on the GMFM-66 scale. Parents reported improvement in behavior, speech development, and a decrease in spasticity in children with CP. G-CSF therapy was well-tolerated. No side effects were observed during the study.

Conclusions: Our preliminary report suggests that G-CSF treatment improves motor and mental function in patients with CP. Further studies are needed to confirm these observations.

Key words: Granulocyte colony-stimulating factor, children, cerebral palsy

*Corresponding author:

Wojciech Kułak

Department of Pediatric Rehabilitation and Center of Early Support for Handicapped Children "Give a Chance", Medical University of Białystok, 17 Waszyngtona street, 15-274 Białystok, Poland

e-mail: kneur2@wp.pl

Received: 22.03.2017

Accepted: 16.05.2017

Progress in Health Sciences

Vol. 7(1) 2017 pp 78-82

© Medical University of Białystok, Poland

INTRODUCTION

Cerebral palsy (CP) is a type of non-progressive brain disorder resulting from various brain injuries that occurred in the period from conception to 1 month after childbirth. Its main clinical manifestations are motor dysfunction, abnormal posture, and often blindness, deafness, epilepsy, mental retardation, and other symptoms. The causes of spastic CP include periventricular leukomalacia, cerebral dysplasia, hypoxia and intrapartum asphyxia, intracranial hemorrhage, and multiple other factors [1].

According to statistics, 1.5–2.5 children per 1000 of the population in developed countries have spastic CP, and this number can be even higher in developing countries [2]. Such a high incidence has placed a heavy burden on families and society [3]. At present, the treatment of children with spastic CP is limited to traditional methods, including physical therapy, rehabilitation training, language training, orthopedic surgery, denervation, and intramuscular injection of botulinum toxin, and other symptomatic treatment, but the effects are unsatisfactory [4]. In recent years, cell transplantation in the treatment of spastic CP has resulted in positive effects in both animal experiments and clinical studies [5–8].

The beneficial effects of granulocyte colony-stimulating factor (G-CSF) were also described for skeletal muscle disorders [9].

Hara et al. [9] showed that G-CSF and its receptor play important roles in muscle development and regeneration. Stratos and his coworkers [10] found that after a blunt muscle injury in animals, administration of G-CSF increased muscular regeneration by satellite cell proliferation and decreased apoptosis.

G-CSF is a hematopoietic cytokine, widely used for the mobilization of hematopoietic stem cells from bone marrow and to treat neutropenia [11,12]. In 2014, G-CSF was tested in an animal model of DMD, the mdx mouse [13]. It was found that treated mdx mice had a higher number of normal muscle fibers compared with untreated mdx mice. Treated mice had 62% of normal muscle fibers and reduced inflammation.

G-CSF induces, directly or through an increase in circulating stem cells, the production of many growth factors (e.g.: insulin-like growth factor 1, epidermal and transforming growth factors, and cytokines) and may have other methods of action on the system of musculature, vessels, and nerves yet to be described [14]. The mechanism of action of G-CSF may also include enhanced successful divisions of satellite stem cells, as recently reported by Canadian researchers [15].

In our previous case reports [16,17], we evaluated the safety and effectiveness of G-CSF in a patient with tetraplegia caused by a cervical

hyperextension injury and in a patient with spastic paraparesis due to kyphoscoliosis. In the patient with spastic tetraplegia, G-CSF 5 µg/kg was administered subcutaneously daily for 5 days per month for 3 months, again after 6 months, and again after 10 months. After this treatment, the boy could sit improperly and walk with assistance. In the patient with spastic paraparesis, clinical examination revealed increased muscle strength in the upper limbs and decreased spasticity in the lower limbs between baseline and day 90 and day 180.

To our best knowledge, there have not been any published studies reporting the use or effects of G-CSF in children with CP.

The purpose of this open trial was to evaluate the efficacy and safety of G-CSF treatment in children and adolescents with CP.

MATERIALS AND METHODS

Ethical approval for the study was obtained from the ethics committee of the Medical University of Bialystok (R-1-002/87/2014). Written informed consent was obtained from the families before study-related procedures.

Study design

A prospective, non-randomized clinical trial assessed the efficacy and safety of G-CSF treatment in patients with CP.

Participants

We enrolled six patients with CP under the care of our department. Inclusion criteria were: patients aged 3-15 years with CP. Children were excluded if surgical interventions or medication changes that might affect motor function were scheduled during the study period; surgery, fractures occurred in the six preceding months; acute inflammation of the musculoskeletal system, and refractory epilepsy. Three children had epilepsy, three children had severe mental retardation, and four patients were classified at V level of the GMFCS. Details are shown in Table 1.

Measures

Gross Motor Function Measure-66 is a standard method of observation, developed to measure changes in motor function over time in children with CP [18]. This test is used in clinical practice and research. Gross Motor Function Measure-66 was used before and after therapy.

The Gross Motor Function Measure-66 (GMFM-66) is a measure of motor function and contains 66 items. Items are grouped into five dimensions: (A) lying and rolling; (B) sitting; (C) crawling and kneeling; (D) standing; and (E) walking, running, and jumping. Each subject is instructed to continue through all domains

according to his/her ability and some items reportedly can be omitted without affecting validity. The GMFM-66 is a valid tool for functional measures in non-ambulatory children.

The GMFM-66 has been validated in children with CP from 5 months to 16 years.

Table 1. Description of children eligible for treatment with granulocyte colony stimulating factor

Sex	Age (in years)	Type of cerebral palsy	Epilepsy	Level of mental retardation	GMFCS level
Girl	3	Spastic paraplegia	Yes	Moderate	III
Girl	15	Spastic paraplegia	No	Mild	V
Boy	3	Spastic paraplegia	Yes	Severe	V
Boy	8	Spastic paraplegia	No	Severe	V
Boy	12	Spastic paraplegia	No	Severe	V

Outcome Measures

G-CSF (5µg/kg body/day) was given subcutaneously in patients with cerebral palsy, during the 1st, 2nd, 3rd, and 6th month.

We used GMFM-66 to measure motor function before and after each cycle of therapy.

We also asked parents to answer the following questions:

- Did you observe any change in the functional status in your child?
- Did you notice new skills in your child?
- Did you notice improvement of daily activities?
- Did you see an increase in muscle strength in your child?
- Is your child more alert or speaks more?

G-CSF administration

G-CSF (5µg/kg/body/day) was administered subcutaneously for 5 consecutive days during the 1st, 2nd, 3rd, 6th, and 12th month (Amgen). We decided to apply a half G-CSF dose using for neutropenia. During each cycle of G-CSF administration in our department, rehabilitation was also applied.

Safety

Laboratory Investigations which included a full blood count, biochemistry: CRP, creatinine, glucose, Electrolytes: Na, K, Cl, Ca, Mg, fibrinogen, kaolin-kephalin time, prothrombin time, creatine kinase (CK), and urine [Laboratory of the Medical University Children Hospital] were performed.

Blood was collected before G-CSF administration and on the 5th day of each treatment cycle.

Abdominal ultrasonography with a spleen measurement was done before and after G-CSF administration. Side effects of G-CSF treatment were evaluated during each cycle of treatment. Electrocardiographic records were also performed.

RESULTS

In all children with CP treated with G-CSF, we observed improvement in the motor function based on the GMFM-66. The greatest improvement in physical activity was observed after the 1st, 2nd and 3rd cycles of G-CSF administration. Details are shown in Table 2.

Table 2. Changes in the scale on the GMFM-66 in children with cerebral palsy after the administration of granulocyte colony stimulating factor

Patient initials	GMFM I	GMFM II	GMFM III	GMFM IV
BW	3.80%	13.60%	9.40%	11.00%
RA	45.20%	42.16%	42.35%	
TF	3.10%	3.45%	4.96%	
DN	26.16%	25.82%	26.56%	
LA	1.17%	1.17%	15.28%	

GMFM - Gross Motor Function Measure

Parents reported that after G-CSF administration their children had:

1. Increased physical activity;
2. Increased endurance during exercises;
3. Decreased spasticity after about a week of G-CSF administration;
4. Improving communication skills;
5. Development of speech;

6. Children were more alert and learned more easily.

No serious adverse events after G-CSF administration were reported by the parents. White blood cell count increased on the 5th day over 20,000 after each G-CSF administration as a reaction to drug application. This returned to normal after four days once treatment was stopped. Red blood count, platelets, CRP, creatinine, glucose, electrolytes (Na, K, Cl, Ca, Mg), fibrinogen, and prothrombin time were in the normal range. Electrocardiographic records were normal.

DISCUSSION

In all patients with CP, we observed improvement in motor function on the GMFM-66. Parents reported positive effects of G-CSF administration. They also observed increased physical activity in children, decreased spasticity, as well as better communication and speech development. G-CSF treatment was well tolerated. No serious adverse events after G-CSF administration were reported by the parents.

Although stem-cell therapy is still in its beginning stages, there are more and more observations of the positive effects of it and G-CSF treatment in patients with neurological diseases [19]. G-CSF increases the proliferation of satellite cells, with transformation into myotubes and muscle fibers, and promote of muscle regeneration [9,21]. These results may point to the general activation of the entire system of cellular regulation rather than a specific target.

It has been shown that G-CSF decreases inflammatory processes and acts positively on peripheral nerve regeneration during the course of muscular dystrophy. This effect was observed in Simões's study on mdx mice [13]. The authors suggest that besides nerve regeneration, G-CSF promotes a favorable microenvironment for axonal regeneration, thereby slowing the progression of DMD. Other authors also indicated that in animal models G-CSF is important for skeletal myocyte development and regeneration [9,20,21].

Similar to our study, Rah et al. [22] in a randomized, double-blind study assessed the neuroregenerative potential of G-CSF followed by infusion of mobilized peripheral blood mononuclear cells (mPBMCs) in children with CP. G-CSF was administered for 5 days, then mPBMCs were collected by apheresis and cryopreserved. They evaluated the efficacy of treatment by using neurodevelopmental tests and neuroimaging studies. The authors observed neurodevelopmental improvement in the patients receiving G-CSF followed by mPBMC.

According to Gonzales-Portillo et al. [23], two major modes of action are involved in stem

cell-mediated functional recovery in ischemic brain injury: cell replacement and the by-stander effect. Molecular neurorestorative mechanisms include neurogenesis, angiogenesis, synaptogenesis, and trophic factor secretion. Furthermore, stem cells serve as a biobridge for the initiation of endogenous repair mechanisms.

CONCLUSIONS

Our preliminary report suggests that G-CSF therapy was well tolerated by patients with CP. The rating scale of motor functions on the GMFM-66 and parents' subjective evaluations suggest the influence of G-CSF on the physical activity and mental development in children with CP. We recommend further studies to assess the efficacy and safety of G-CSF in patients with CP in a larger population group and other types of CP.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. Shevell MI, Majnemer A, Morin I. Etiologic yield of spastic cerebral palsy: a contemporary case series. *Pediatr Neurol.* 2003;28(5):352–9.
2. Paneth N, Hong T, Korzeniewski S. The descriptive epidemiology of spastic cerebral palsy. *Clin Perinatol.* 2006;33(2):251–67.
3. Liu X, Fu X, Dai G, Wang X, Zhang Z, Cheng H, Zheng P, An Y. Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. *J Transl Med.* 2017 Feb 24;15(1):48.
4. Holt RL, Mikati MA. Care for child development: basic science rationale and effects of interventions. *Pediatr Neurol.* 2011;44(4):239–53.
5. Sharma A, Sane H, Gokulchandran N, Kulkarni P, Gandhi S, Sundaram J, Paranjape A, Shetty A, Bhagwanani K, Biju H, Badhe P. A clinical study of autologous bone marrow mononuclear cells for spastic cerebral palsy patients: a new frontier. *Stem Cells Int.* 2015;2015:905874.
6. Jin JL, Liu Z, Lu ZJ, Guan DN, Wang C, Chen ZB, Zhang J, Zhang WY, Wu JY, Xu Y. Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. *Curr Neurovasc Res.* 2013;10(1):11–20.
7. Shroff G, Gupta A, Barthakur JK. Therapeutic potential of human embryonic stem cell transplantation in patients with spastic cerebral palsy. *J Transl Med.* 2014;12:318.
8. Dai G, Liu X, Zhang Z, Yang Z, Dai Y, Xu R. Transplantation of autologous bone marrow

- mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. *Brain Res.* 2013;1533:73–9.
9. Hara M, Yuasa S, Shimoji K, Onizuka T, Hayashiji N, Ohno Y, Arai T, Hattori F, Kaneda R, Kimura K, Makino S, Sano M, Fukuda K. G-CSF influences mouse skeletal muscle development and regeneration by stimulating myoblast proliferation. *J Exp Med.* 2011; 208(4):715-27.
 10. Stratos I, Rotter R, Elpel C, Mittlmeier T, Vollmar B. Granulocyte-colony stimulating factor enhances muscle proliferation and strength following skeletal muscle injury in rats. *J Appl Physiol.* 2007;103:1857-63.
 11. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. *Blood* 1999;78:2791-808.
 12. Metcalf D. Hematopoietic cytokines. *Blood* 2008;111:485-91.
 13. Simões GF, Benitez SU, Oliveira ALR. Granulocyte colony-stimulating factor (G-CSF) positive effects on muscle fiber degeneration and gait recovery after nerve lesion in MDX mice. *Brain Behav.*2014;4(5):738-53.
 14. Ruozi B, Belletti D, Bondioli L, De Vita, A, Forni F, Vandelli, M. Tosi G. Neurotrophic factors and neurodegenerative diseases: a delivery issue. *Int Rev Neurobiol.* 2012; 102: 207-47.
 15. Dumont NA, Rudnicki MA. Targeting muscle stem cell intrinsic defects to treat Duchenne muscular dystrophy. *NPJ Regen Med.* 2016; 1:16006.
 16. Okurowska-Zawada B, Kułak W, Sienkiewicz D, Paszko-Patej G, Dmitruk E, Kalinowska, A. Safety and efficacy of granulocyte colony stimulating factor in a patient with tetraplegia caused by cervical hyperextension injury: a case report. *Prog Health Sci.* 2014;4(2):181-4.
 17. Sienkiewicz D, Kułak W, Okurowska-Zawada B, Wojtkowski J, Paszko-Patej G, Dmitruk E, Kalinowska A, Okulczyk K. Potential beneficial effects of granulocyte colony-stimulating factor therapy for spastic paraparesis in patient with kyphoscoliosis: a case report. *Neuropediatrics* 2014;45(5):325-7.
 18. Russell DJ, Rosenbaum PL, Wright M, Avery LM. Gross Motor Function Measure (GMFM-66 & GMFM-88) User's Manual. London: Mac Keith Press;2013.
 19. Kułak W, Okurowska-Zawada B, Sienkiewicz D, Paszko-Patej, G. Cell therapy: a New hope for treatment of cerebral palsy? *Prog Health Sci.* 2012;2(2):174-80.
 20. Sienkiewicz D, Kułak W, Okurowska-Zawada B, Paszko-Patej G, Kawnik K. Duchenne muscular dystrophy: Current cell therapies. *Ther Adv Neurol Disord.* 2015;8(4):166-77.
 21. Hayashiji N, Yuasa S, Miyagoe-Suzuki Y, Hara M, Ito N, Hashimoto H, Kusumoto D, Seki T, Tohyama S, Kodaira M, Kunitomi A, Kashimura S, Takei M, Saito Y, Okata S, Egashira T, Endo J, Sasaoka T, Takeda S, Fukuda K. G-CSF supports long-term muscle regeneration in mouse models of muscular dystrophy. *Nat Commun.* 2015;13(6):6745.
 22. Rah WJ, Lee YH, Moon JH, Jun HJ, Kang HR, Koh H, Eom HJ, Lee JY, Lee YJ, Kim JY, Choi YY, Park K, Kim MJ, Kim SH. Neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in children with cerebral palsy: a randomized, double-blind, cross-over study. *J Transl Med.* 2017 Jan 21;15(1):16.
 23. Gonzales-Portillo GS, Reyes S, Aguirre D, Pabon MM, Borlongan CV. Stem cell therapy for neonatal hypoxic-ischemic encephalopathy. *Front Neurol.* 2014 Aug 12;5:147.