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A study on effect of filgrastim in severe leucopenia associated with hemorrhagic gastroenteritis in dogs

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Abstract
Filgrastim is a recombinant Methionyl Human Granulocyte Colony-Stimulating Factor (r-metHuG-CSF) analog used to stimulate the proliferation and differentiation of granulocytes in humans. Young pups suffer from hemmoragic gastroenteritis (etiology may be viral like Canine Parvovirus or Canine Distemper Virus, bacterial like *E.coli* or parasitic) mainly associated with severe leukopenia and neutropenia. Neurtrophils being first line of defense against sepsis, decreased count may lead to increased mortality. In the present investigation eleven dogs with severe leukopenia and neutropenia when treated with Filgrastim @ 10 µg/ kg b.wt. s.c daily for three days along with other supportive treatment, was found to be effective in correcting the hematological picture within 48 hours of last dose of treatment and all the dogs showed fast recovery uneventfully.

Keywords: Leukopenia, neutropenia, hemorragic gastroenteritis, filgrastim

Introduction
Hemorrhagic gastroenteritis is most common cause of fatality in young pups. It's clinical course usually includes a peracute onset of clinical signs that can progress rapidly to death in absence of appropriate therapy. It is most commonly caused by Canine Parvovirus, Canine Distemper Virus, Bacterial infections like *E.coli* and *Colistridium* spp. etc. Out of these, canine parvovirus remains the most significant viral cause of enteritis in dogs less than 6 months of age (Appel *et al.*, 1979) [1]. Canine parvovirus has a predilection for rapidly dividing cells, making the granulocyte precursor pool in the bone marrow a prime target for destruction in young pups. Neutropenia frequently occurs following Canine Parvoviral infection along with other several different causes (Macartney *et al.*, 1984) [8]. In addition, loss of neutrophils may occur through the damaged gastrointestinal tract (Fulton *et al.*, 1991) [5].
Neutrophils are most important cells required for immune system to combat with the bacterial infection. So prolonged neutropenia greatly increases the risk of death, due to sepsis. Hence strategies are required to shorten the duration of neutropenia in dogs. The use of granulocyte-colony stimulating factor, a cytokine and growth factor that potently stimulates neutrophil production and release from the bone marrow, has been advocated for treatment of parvovirus-induced neutropenia (Kraft & Kuffer, 1995) [7]. Haematopoietic growth factors that stimulate leukocyte generation and or differentiation are important in the response to infection (Rewerts & Henry, 1998) [12]. The primary clinical use of G-CSF in humans is mainly for management of chemotherapy-induced neutropenia (Czygier *et al.*, 2007) [3]. In veterinary medicine, rcG-CSF has also been evaluated for use in management of chemotherapy-induced neutropenia (Ogilvie *et al.*, 1992) [11]. Filgrastim is a non-glycosylated, 175 amino acid containing protein, which is produced recombinantly by *E. coli.* Filgrastim has a molecular weight of 18.8 kDa and it regulates the production and release of functional neutrophils from bone marrow within 24 hours of administration. Filgrastim results in increase in peripheral blood neutrophil counts with minor increase in monocytes (Areshkumar *et al.*, 2017) [2]. Therefore, we conducted a study to determine whether treatment with rcG-CSF in dogs, with neutropenia and leukopenia suffering from hemorragic gastroenteritis, was effective in correcting haematological abnormalities. We hypothesized that rcG-CSF treatment would stimulate more rapid recovery of white blood cell (WBC) and neutrophil counts.

Materials and Methods
Eleven dogs suffering from hemmoragic gastroenteritis, were included in the present study. Clinical signs observed were vomition and diarrhoea with blood present in vomitus and feces. The dogs were having different degrees of dehydration.

The blood sample (2ml) was collected in a vial containing EDTA and subjected to haematological examination (haemoglobin, packed cell volume, total and differential leucocyte count, thrombocyte count) with the help of haemato-analyser (MS4Se-melet Schloesing Laboratories-France Affected dogs were treated with Inj. Filgrastim @ 10 µg / kg b.wt. subcutaneously once daily (Morris and Dobson, 2001) [10] for three days along with supportive therapy (intravenous fluid, antibiotics and antiemetics) as given in table 1. The blood samples were collected again on day 3 post treatment and data was analysed and interpreted by paired t test.

Table 1: Supportive therapy given to the animals (depending on clinical condition)

Drug	Dose (per Kg B.wt)
Inj. Lactate ringer solution	15ml
Inj. Dextrose 5%	15ml
Inj. Metronidazole	25mg
Inj. Astymin ® (cocktail of aminoacids)	1ml
Inj. Ondansetron	0.1 mg
Inj. Ranitidine	2 mg
Inj. Pantaprazole	1 mg

Results and discussion

Hemorrhagic gastroenteritis in dogs is a life-threatening problem. Although the dogs which were not vaccinated properly suffered the most, but occurrence of the disease was also noticed in vaccinated dogs and the leukocytes count was very low. Greene and Decaro (2012) [6] reported that those pups dying from Parvovirus disease generally have TLC equal to or less than 1030 cells/µl and have persistent lymphocytopenia, monocytopenia and eosinopenia within the first 3 days of hospitalization. Haematological parameters revealed marked leukopenia with neutropenia in all the cases at day of presentation. WBC count was found to be 1.99 ± 0.23 thousand per cumm and neutrophil count was found to be 1.03 ± 0.14 thousand per cumm. Remaining haematological findings are given in table 2. To overcome this critical situation in the present study Inj. Filgrastim at the dose rate of 10 µg/kg body weight by S/C route was administered daily for three days. After 72 hours of the injection, the leucocyte count found to be elevated significantly by 8.064 ± 1.80 thousand per cumm**.** There was significant difference in neutrophil count by 5.10 ± 0.79 thousand per cumm and lymphocyte count by 2.97 ± 0.95 thousand per cumm of day 1 and day 3 post treatment samples because filgrastim mainly acts on bone marrow and it increases production of leucocytes.. Although there was no significant increase in hemoglobin, RBC, PCV and Platelet count as shown in Table3 as there is no effect of filgrastim on erythropoiesis or thrombocyte production. Rewerts *et al*., 1998 [12] and Mischke *et al*., 2001 [9] did not find any improvement in neutrophil counts or duration of hospitalization in treated animals when compared to untreated animals in cases. However Kraft & Kuffer (1995) [7], Ogilvie *et al*., (1992) [11], Duffy *et al*., (2009) [4] and Areshkumar *et al*., (2017) [2] found significantly increased neutrophil counts compared to control dogs suffering from neutropenia. So, from the present study we concluded that administration of Filgrastim may improve the survival rate if it is administered at the early stage of the disease along with other supportive therapy as uneventful recovery was received in all the treated cases.

Table 2: Hematological pictures of dogs suffering from Hemorrhagic gastroenteritis (n=11)

Parameter	Case No.	I		II		III		IV		V		VI		VII		VIII		IX		X		XI	
	Day	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
Hemoglobin (g/dL)		9.6	9.1	12.6	11.6	10.3	9.8	13.9	14	10.9	10.2	11.6	11.8	9.9	10.2	8.1	9.8	12	9.6	12.4	11.6	12.4	10.8
PCV (%)		29	32	34.6	32	32.9	30.8	41	43	34	32	32	33	31	33	31	36	39	34	41	38	41	35
RBC (million/mm^3)		4.76	5.26	5.65	5.60	4.66	4.89	5.25	6.20	5.08	5.04	7.36	7.45	6.25	6.15	5.23	5.63	6.36	5.46	7.21	6.32	6.8	5.83
WBC (cells /µL)		3100	7000	1700	6500	3200	6100	1200	5900	1680	9020	2100	8600	2130	7600	2980	6700	1500	12900	1040	17420	1340	22950
Platelets (lakhs/µl)		3.78	4.65	5.77	5.96	7.01	6.80	2.31	3.51	6.01	8.30	6.52	6.26	4.96	5.21	1.09	2.31	3.26	3.45	7.21	1.17	3.59	2.89
Neutrophils (cells/mm^3)		48	63	30	62	27	56	47	62	59	73	48	65	39	59	86	84	57	64	66	53	73	53
Lymphocytes (cells/mm^3)		49	35	65	37	71	42	48	35	37	20	50	31	55	38	11	15	37	27	29	37	23	39
Monocytes (cells/mm^3)		1	1	3	1	1	1	2	1	4	7	2	1	6	3	3	1	6	9	5	10	4	8
Eosinophils (cells/mm^3)		2	1	2	0	1	1	3	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0

Table 3: Comparison of different hematological parameters of blood samples of Day1 and Day3 Post treatment (n=11)

Parameter	Day1	Day 3 Post Treatment
Hemoglobin (g/dL)	11.25 ± 0.50	10.77 ± 0.42
PCV (%)	35.13 ± 1.37	34.43 ± 1.06
RBC (million/mm^3)	5.87 ± 0.29	5.8 ± 0.22
WBC (thousand cells/µL)	1.99 ± 0.23^a	10.06 ± 1.66^b
Neutrophils (cells/mm^3)	1.03 ± 0.14^c	6.13 ± 0.82^d
Lymphocytes (cells/mm^3)	0.96 ± 0.15^e	3.93 ± 0.88^f
Platelets (lakhs/µl)	4.68 ± 0.61	4.59 ± 0.65

*Mean (±SE) bearing different superscripts (a, b) differ significantly ($p< 0.05$) before and after treatment
*Mean (±SE) bearing different superscripts (c, d) differ significantly ($p< 0.05$) before and after treatment
*Mean (±SE) bearing different superscripts (e/f) differ significantly ($p< 0.05$) before and after treatment
No significant difference between Hb, PCV, RBC and Platelets count of Day1 and Day3 post treatment

References

1. Appel MJ, Scott FW, Carmichael LE. Isolation and immunisation studies of a canine parco-like virus from dogs with haemorrhagic enteritis. Veterinary Record 1979;105:156-159.
2. Areshkumar M, Vijayalakshmi P, Venkatesa Perumal S, Selvi D. Effect of Filgrastim in a Severe Leucopenia associated Parvoviral Enteritis in Rottweiler.

International Journal of Current Microbiology and Applied Sciences 2017;6(4):1060-1065.
3. Czygier M, Dakowicz L, Szmitkowski M. The effect of granulocyte colony-stimulating factor (G-CSF) on neutrophil functions in children with neutropenia after chemotherapy in the course of neoplasma. Advances in Medical Sciences 2007;52:143-146.
4. Duffy A, Dow S, Ogilvie G, Rao S, Hackett T. Hematologic improvement in dogs with parvovirus infection treated with recombinant canine granulocyte-colony stimulating factor. Journal of Veterinary Pharmacology and Therapeutics 2009;33:352-356.
5. Fulton R, Gasper PW, Ogilvie GK, Boone TC, Dornsife RE. Effect of recombinant human granulocyte colony-stimulating factor on hematopoiesis in normal cats. Experimental Hematology 1991;19:759-767.
6. Greene CE, Decaro N. Canine viral enteritis. In: Greene CE, editor. Infectious diseases of the dog and cat 4th ed. St. Louis: Elsevier 2012, 67-80.
7. Kraft W, Kuffer M. Treatment of severe neutropenias in dogs and cats with filgrastim. Tierärztliche Praxis 1995;23:609-613.
8. Macartney L, McCandlish IA, Thompson H, Cornwell HJ. Canine parvovirus enteritis 1: clinical, haematological and pathological features of experimental infection. Veterinary Record 1984;115:201-210.
9. Mischke R, Barth T, Wohlsein P, Rohn K, Nolte I. Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on leukocyte count and survival rate of dogs with parvoviral enteritis. Research in Veterinary Science 2001;70:221-225.
10. Morris J, Dobson J. Small animal oncology, Black well sci. ltd. 2001, 288.
11. Ogilvie GK, Obradovich JE, Cooper MF, Walters LM, Salman MD, Boone T.C. Use of recombinant canine granulocyte colonystimulating factor to decrease myelosuppression associated with the administration of mitoxantrone in the dog. Journal of Veterinary Internal Medicine 1992;6:44-47.
12. Rewerts JR, Henry CJ. Veterinary uses of recombinant human granulocyte colony stimulating factor Part II: Infectious diseases. Compendium 1998;20:823-829.