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A Study of Peripheral Blood and Bone Marrow Responses Depends on the Frequency of rhG-CSF Administration in Dogs

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Abstract : The present study evaluated that responses of peripheral and bone marrow depends on the frequency of recombinant human granulocyte colony-stimulating factor (rhG-CSF) administration in dogs. The rhG-CSF has been revealed that have a beneficial effect for dogs with myelosuppression secondary to chemotherapy or radiation but there were no studies about the frequency of administration in dogs. In this research, rhG-CSF was administrated 5 μg/kg subcutaneously for each two-dogs group as follows: (1) every day for trial, (2) every other day for trial, (3) every third day for trial. The peripheral blood analysis including direct microscopic differential counts of one hundred cells was performed every day. Bone marrow aspiration was performed before administration of rh G-CSF, on the day of 0, 3, 9 and when the WBC counts were decreased within the normal range (day 12 or 13). Rh G-CSF was well-tolerated and showed no side effects in all dogs. According to the present study, 5 μg/kg administration of rhG-CSF have cell-specific, frequency-related effect on bone marrow and peripheral blood. Furthermore, the effects of rhG-CSF administration on bone marrow sustained during the study and prolonged at least 3 days after discontinuing of rhG-CSF treatment.

Key words **:** rhG-CSF, dog, leukopenia.

Introduction

Colony stimulating factors (CSFs) are glycoproteins that manage hematologic regulation (2,30). These factors can stimulate the hematologic system in two ways. They can augment pluripotent stem cells and progenitor cells, as is the case for interleukin-3 (IL-3), granulocyte macrophage CSF (GM-CSF), and interleukin-6 (IL-6), or they can induce differentiation of proliferative cells into mature cells, which is true for erythropoietin and granulocyte CSF (G-CSF) (28). Because CSFs have the potential to provide benefit to patients with many clinical conditions, they have been used in various treatment trials for treating disorders such as cyclic hematopoiesis, drug-induced cytopenia, chemotherapy-induced myelosupression, viral infectious disease, and bone marrow transplantation (6,17,21,27,29). In addition, it has been suggested that CSFs might induce expansion of primed cells that have antitumor effects and provide host defense against infection (37).

G-CSF stimulates proliferation and differentiation of granulocytes in bone marrow, causing mature neutrophils to move from bone marrow into the circulation, and promoting phagocytosis, chemotaxis, and oxidative activities in mature neutrophils (7,10,15). It is produced by epithelial cells, endothelial cells, macrophages, bone marrow stromal cells, fibroblastsand mature neutrophils in reaction to various proinflammatory mediators, e.g., tumor necrosis factor, interleukin-1, interleukin-3, interferon-γ and granulocyte macrophage colony-stimulating factor (7). Recombinant human G-CSF (rhG-CSF) has been produced (12) and has been commercially available since the early 1990s (12,23). It has been revealed to provide beneficial effects to human patients with cytopenia (12,23) and to dogs with myelosuppression secondary to chemotherapy (23) or radiation therapy (18,29), or with cyclic neutropenia (5). Clinical use of rhG-CSF has been widely adopted owing to the fact that it is well-tolerated (with minimal side effects) in humans and animals (16,37). Recombinant canine G-CSF (rcG-CSF) has also been produced for research purposes (22,24,25). However, it is not acceptable for clinicians since it is not commodified (21).

In humans, a measurable increase in total leukocytes is observed approximately 12 hours after a single injection of rhG-CSF, which is attributable to the release of granulocytes from a storage pool in bone marrow to the peripheral blood (14). Previous clinical studies suggest that rhG-CSF causes a marked decrease in the transit time of myeloid cells, as well as amplification of progenitor cells in bone marrow (1). By

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contrast, most published studies of rhG-CSF in veterinary medicine are focused on its adverse effects or clinical application in a canine myelosuppression model. In addition, no research has been performed concerning the short-term response of bone marrow to rhG-CSF administration in normal dogs, although a few studies have evaluated bone marrow changes after long-term treatment with rhG-CSF (8).

The production of antibodies is the most common adverse effect of rhG-CSF administration in animals. Repeated administration of high-dose rhG-CSF or long-term treatment with rhG-CSF causes the production of antibodies that neutralize endogenous canine G-CSF (19). Therefore, clinicians are often reluctant to perform long-term treatment or give high-dose injections of rhG-CSF to their patients. Given the lack of data regarding the short-term effects of rhG-CSF on bone marrow, it is difficult to develop treatment plans (regarding frequency of administration) for leukopenia patients.

Although the issue of whether peripheral blood and bone marrow responses to rhG-CSF depend on the frequency of injections has not been studied, dose-related effects on leukocyte counts of peripheral blood have been investigated (19). Administration of G-CSF every 2 days has been shown to prevent anaphylaxis and febrile neutropenia in humans (36), but similar research has not been performed in dogs. In general, a dose of 5 μg/kg/day is suggested for dogs with neutropenia (3). However, daily administration of rhG-CSF has been hard to achieve clinically, owing to cost. In this situation, clinicians must alter the frequency of rhG-CSF administration. However, the lack of data available on hematologic changes in response to frequency makes this task difficult.

Therefore, the purpose of the present report was to examine the hematologic changes that occur in response to different frequencies of rhG-CSF administration and the short-term effects of rhG-CSF administration on bone marrow in normal dogs.

Materials and Methods

Animals

Six adult Beagles (mean weight 12.58 kg; three intact female dogs and three intact male dogs) were used in this study (Table 1). All dogs were housed in separate cages and regularly provided with commercial dry food and chlorinated tap water. They were screened for evidence of disease using physical examination, complete blood cell count, serum chemistry, and confirmed negative infection for *ehrlichia canis*, heartworm, *anaplasma* and *borrelia burgdorferi* (4DX kit, Idexx, USA). All dogs were treated in accordance with the guidelines approved by the Institutional Animal Care and Use Committees (IACUC) of Gyeongsang National University.

Recombinant human G-CSF (rhG-CSF)

The rhG-CSF used in this study was prepared and provided by CJ Cheiljedang Corporation (Leukokine®, South Korea). The dogs were separated into three groups and rhG-CSF was administered subcutaneously at a dose of 5 μg/kg to each group as follows: (1) every day for 10 days (10 times in total), (2) every other day for 11 days (six times in total), and (3) every third day for 10 days (four times in total).

Table 1. Signalment and group of 6 laboratory dogs

Group	Duration of administration	Dog Number	Breed	Sex	Mean body weight
1	Every day	1-1	Beagle	Male	12
		1-2	Beagle	Female	11.5
2	Every other day	2-1	Beagle	Female	14.7
		2-2	Beagle	Female	9.5
3	Every third day	3-1	Beagle	Male	13.6
		3-2	Beagle	Male	14.2

Peripheral blood analysis

Peripheral blood samples were obtained from the jugular vein on each day of the study. Before rh G-CSF administration, the samples were collected into EDTA-containing tubes, and complete blood cell counts analysis were performed between 5 and 8 p.m. using an automatic blood cell counter (Celltacα MEK-6450K, Nihon Kohden, Tokyo). Differential blood cell counts were performed per 100 cells for each sample taken. Band cell count was measured daily by directly counting cells in the peripheral blood under the microscope.

Bone marrow aspiration

Bone marrow aspiration was performed before administration of rhG-CSF on days of 0, 3, and 9 of the study. Additional bone marrow aspiration was performed when the white blood cell (WBC) counts were decreased within the normal range.

Prior to aspiration, dogs were sedated by intravenous injection of medetomidine (Domitor, Zoetis, Seoul). The sample was collected from both sides of the humerus and femur with a 13-gauge bone marrow biopsy needle (Medical Device Technologies INC, Angiotech, USA). The site was cleansed aseptically using povidone-iodine and alcohol. A 10-mL syringe containing anticoagulant (heparin) was used to collect the bone marrow, which was smeared on a slide and stained with Diff-Quick (Hemacolor®, Merk Millipore, USA) .

Myeloid:erythroid (M:E) cell ratios were calculated using at least 500 bone marrow cells, and cells were classified as maturing cells, proliferating cells, lymphocytes, eosinophils, or other cells.

Statistical analysis

Statistical analysis was performed using the SPSS 12.0 program. The Kruskal-Wallis test and the Mann-Whitney U test were used to compare counts of leukocytes, granulocytes, eosinophils, lymphocytes, monocytes, and the M:E ratio

of bone marrow among groups. For all analyses, a value of $P < 0.05$ was considered statistically significant.

Results

White blood cells (WBC)

Total leukocytes

Twenty-four hours after rhG-CSF administration, WBC count of all dogs increased. The changes in WBC count results are shown in Fig 1. All dogs in group 1 experienced sustained leukocytosis and an increase in WBC count for most of the study period; a steady and rapid increase was observed during the trial. On the day 10 of the study, leukocyte counts were six- to seven-fold higher than at pre-trial baseline. WBC counts of the dogs in group 2 increased on the second day of rhG-CSF injection and decreased the day after. However, both dogs in group 2 showed a marked increase in WBC count on day 9, three to four times higher than the pretreatment level. Leukocyte counts of both dogs in group 2 exceeded the established reference range (6,000-17,000/μl) during the trial. By contrast, most WBC counts were back within the normal range on the third day after rhG-CSF administration in group 3, although they increased again to three-fold to four-fold higher than baseline on the day after the last rhG-CSF injection. The leukocyte count of all groups decreased after discontinuation of rhG-CSF and returned to within the reference range 2 days later. Comparing the average WBC counts among groups suggests that leukocytosis is related to the frequency of rhG-CSF administration (Fig 2); group 1 showed a striking increase in leukocytosis compared to the other groups. Overall, rhG-CSF was well tolerated and no adverse effects were observed.

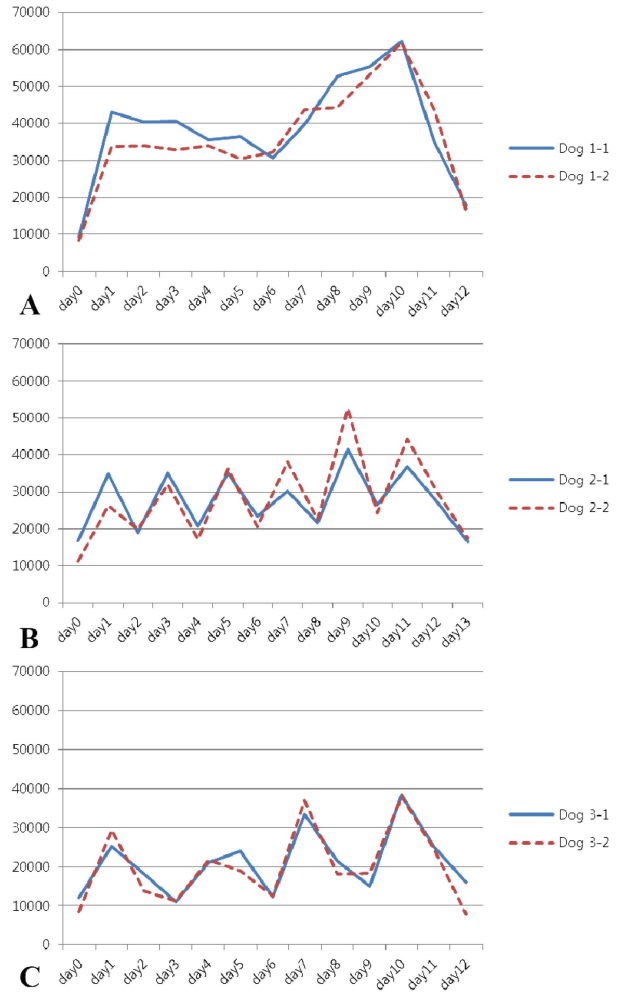

Fig 1. Comparison among three groups of total leukocyte counts of dogs given 5 μg rhG-CSF/kg subcutaneously (A: Group 1, B: Group 2, C: Group 3). A: Daily administration of rhG-CSF leads to increase of total leukocyte count continuously. B: Leukocyte count of group 2 was not showed sustained increase but showed exceeding the established reference range (6000-17000/μl). C: Total leukocyte of group 3 showed decreased within the normal range on the third day after G-CSF administration. *Reference range: 6,000-17,000/μl.

Granulocytes

Granulocyte count showed a similar trend to total leukocyte count between groups. Granulocyte count was significantly different among the three groups ($P < 0.05$) (Table 2).

Lymphocytes

Lymphocyte count was not significantly different between groups. In group 1, however, lymphocyte count markedly increased (approximately six-fold compared with pre-trial levels) on day 2 and on day 10 (Table 2).

Eosinophils

Changes in eosinophil levels were significantly different

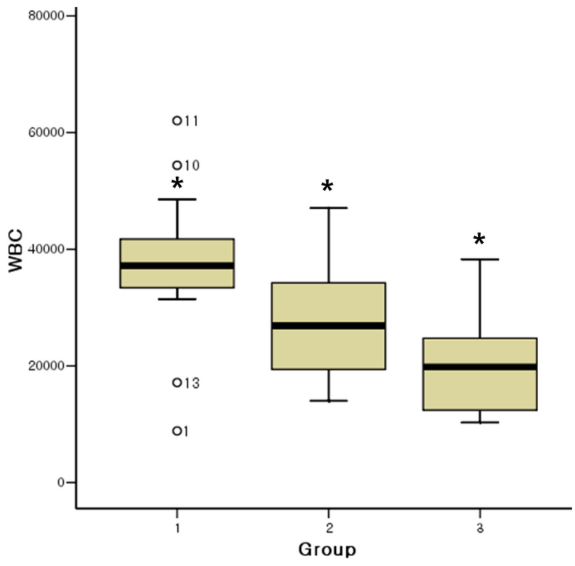

Fig 2. Box and whisker diagram for comparison of total leukocyte counts among three groups. Total leukocyte count showed a frequency-dependent tendency. *There was a significant difference among three groups (P value < 0.05).

Table 2. Changes in granulocyte, lymphocyte, eosinophil, monocyte in six dogs

		day0	day1	day2	day3	day4	day5	day6	day7	day8	day9	day10	day11	day12	day13
Granulocyte (cells/μl)	Dog 1-1	6700	39200	27200	35000	29500	31200	27100	35200	47100	49600	48300	31600	15100	
	Dog 1-2	5300	22400	22400	27200	30700	26000	29100	36800	36700	47800	48800	34300	12500	
	Dog 2-1	7900	19800	14100	27700	13600	29100	15200	31200	18400	47800	19200	36200	24400	13200
	Dog 2-2	11900	26200	13900	27700	15600	26700	17900	24600	17100	33900	20000	27700	21200	10600
	Dog 3-1	9000	19600	12500	7800	16900	18800	8600	24900	15800	9800	28600	18600	10800	
	Dog 3-2	6300	23500	9200	8200	17900	14400	9300	32800	14100	13200	31100	21100	5400	
Lymphocyte (cells/μl)	Dog 1-1	1900	2400	9000	3500	4100	3500	2300	3000	3400	3300	9800	2400	2000	
	Dog 1-2	2600	7800	8000	3900	1900	3100	2000	4700	5200	3100	9200	6700	3100	
	Dog 2-1	2500	4700	4600	2700	2700	5200	4300	4900	3000	2500	3700	5500	4100	3200
	Dog 2-2	3700	6500	3800	5300	3800	6300	4300	4100	3400	5100	5000	7100	4400	5000
	Dog 3-1	2400	4300	4600	2400	3000	4000	2900	6200	4200	4300	7100	4900	3800	
	Dog 3-2	1700	4400	3300	2500	2800	3600	2700	2600	3200	4200	5100	2500	2000	
Eosinophil (cells/μl)	Dog 1-1	0	100	200	800	400	400	500	300	600	1300	400	300	200	
	Dog 1-2	0	100	200	500	800	600	600	900	800	1500	500	500	200	
	Dog 2-1	300	200	100	700	200	300	100	600	300	1200	400	700	100	0
	Dog 2-2	300	200	400	700	400	400	300	500	400	900	400	400	500	100
	Dog 3-1	100	200	100	100	200	100	0	100	100	200	200	100	100	
	Dog 3-2	100	200	100	200	300	100	100	600	500	400	200	200	0	
Monocyte (cells/μl)	Dog 1-1	600	1400	4000	1400	1500	1300	700	1200	1600	1300	3700	800	500	
	Dog 1-2	600	3400	3300	1400	700	700	600	1400	1700	900	3400	2200	700	
	Dog 2-1	600	1600	1100	900	700	1500	1000	1500	800	1000	900	1900	1200	1000
	Dog 2-2	800	1900	800	1500	900	1600	1000	1100	800	1700	1000	1600	1000	800
	Dog 3-1	600	1200	1000	700	1000	1000	700	2300	1300	700	2600	1400	1300	
	Dog 3-2	400	1300	1200	400	800	900	500	900	800	900	1700	700	400	

Table 3. Changes of band cell count in six dogs during trial (/100cells)

	day0	day1	day2	day3	day4	day5	day6	day7	day8	day9	day10	day11	day12	day13
Dog 1-1	0	2	6	4	2	5	1	4	11	9	3	7	3	
Dog 1-2	0	1	4	8	4	4	1	1	4	6	5	3	0	
Dog 2-1	1	2	1	4	2	4	2	9	4	11	1	5	4	0
Dog 2-2	0	4	2	8	3	6	2	12	5	14	3	8	6	0
Dog 3-1	0	2	0	0	2	1	0	0	2	1	1	1	0	
Dog 3-2	0	0	0	1	3	1	1	1	0	0	2	1	1	

between groups. In groups 1 and 2, a notable increase was observed on day 9. No significant change was observed in group 3 (Table 2).

Monocytes

There was no significant difference in monocyte level between the three groups. Similar to lymphocyte level, a prominent increase (a three-fold increase compared with baseline) was demonstrated on day 2 and day 10 in all groups (Table 2).

Band cells

Band cell changes are shown in Table 3. Band cell count increased in relation to injection frequency.

Bone marrow

Myeloid:erythroid (M:E) cell ratio

M:E ratio increased continuously after repeated rhG-CSF injections in all dogs, with a marked increase observed on day 9 in all groups. M:E ratio was significantly (approximately three-fold) higher in groups 1 and 2, compared with group 3. Hematocrit levels did not change significantly for any group throughout the study, which suggests that the increase in M:E ratio was due to repopulation of myeloid cells in bone marrow and relative hypoplasia of erythroid cells.

Three days after cessation of rhG-CSF injections, when leukocyte counts of the peripheral blood declined into the normal range, the M:E ratio was still higher than at baseline

Table 4. Changes in myeloid:erythroid (M:E) ratio in six dogs by bone marrow aspiration

	day 0	day 3	day 9	Last day*
Dog 1-1	1.81	3.43	5.06	4.5
Dog 1-2	2.29	3.45	6.23	5.3
Dog 2-1	0.97	1.85	2.52	2.4
Dog 2-2	1.61	2.28	6.13	3.14
Dog 3-1	1.22	1.6	2.1	1.45
Dog 3-2	1.29	2.2	2.49	1.93

*Group 1 and Group 3 : Day 12 , Group 2 : Day 13

Table 5. The average increasing rate of myeloid:erythroid (M:E) ratio during the trial in three groups

	Group 1	Group 2	Group 3
day 0	100 (%)	100(%)	100 (%)
day 3	170 (%)	166 (%)	152 (%)
day 9	276 (%)	320 (%)	183 (%)
Last day*	240 (%)	221 (%)	134 (%)

*Group 1 and Group 3 : Day 12 , Group 2 : Day 13

in all groups. On day 12 or 13, M:E ratio was two-fold higher than on day 0, particularly in group 1 and 2 (Table 4 and 5).

Proliferative:maturing or storage cells (P:M) ratio

P:M ratio exhibited no significant changes during the trial. However, the average P:M ratio of group 1 increased approximately two-fold, compared with pretreatment level, in the first 3 days. Conversely, the P:M ratio decreased over the same period in groups 2 and 3. Six days later, the P:M ratio increased in group 2. These findings indicate that daily administration of rhG-CSF is more beneficial than administration every 2-3 days in terms of increasing the immature neutrophil pool in bone marrow (Table 6).

Discussion

This report demonstrates that peripheral WBC counts increase significantly in dogs who receive daily rhG-CSF injections compared with dogs that receive injections every 2 days or every 3 days. Leukocytosis occurred within 24 hours of the first injection in all groups. This initial increase is likely attributable to demargination or release of mature cells from the marrow rather than differentiation of progenitor cells (33). G-CSF can increase leukocyte count by reducing the time taken for progenitor cells to mature. Indeed, granulopoiesis in bone marrow takes 3-5 days under normal physiological conditions in humans, but is shortened to 1 day under G-CSF treatment (20). However, this effect takes at least 2-3 days to detect in peripheral blood (21).

In this study, frequency-dependent eosinophilia was observed, although no significant changes were found in the levels of monocytes, lymphocytes, and other cells. Because eosinophils do not express the G-CSF receptor, there was little correlation between rhG-CSF administration and eosinophila (13). In previous studies, differing results have been reported concerning eosinophil levels after administration of rhG-CSF in canine models (9,29). One study demonstrated that administration of rhG-CSF to healthy humans caused eosinophilia by increasing the number of circulating eosinophils and mobilizing eosinophil granule proteins (33). Furthermore, unlike in man and nonhuman primates, rhGM-CSF was able to cause moderate eosinophilia in dogs (3).

Lymphocyte levels were 3-3.5 times higher on day 2 and day 10 than on day 0 in all dogs, with no evidence of a frequency-dependent increase. Previous reports have demonstrated diverse effects of G-CSF administration on lymphocytes (9,29). In humans, rhG-CSF stimulates mobilization and blastogenesis of lymphocytes (31,32), with a report showing that administration of rhG-CSF enhances lympho-

Table 6. Changes in proliferative cells:maturing cells/Storage cells (P:M) ratio of six dogs by bone marrow aspiration

Dog		Day 0	Day 3	Day 9	Last day
Dog 1-1	proliferative cells (%)	2.6	9	7.2	6.2
	maturing/storage cells (%)	58	65	71.8	69.4
	P:M ratio	0.04	0.14	0.10	0.09
Dog 1-2	proliferative cells (%)	5.4	6	6.4	5.8
	maturing/storage cells (%)	61.2	65.8	75	73.8
	P:M ratio	0.09	0.09	0.09	0.08
Dog 2-1	proliferative cells (%)	5	4.4	4.6	8.8
	maturing/storage cells (%)	40.2	55.2	63.8	57.6
	P:M ratio	0.12	0.08	0.07	0.15
Dog 2-2	proliferative cells (%)	6.6	4.4	5.4	8
	maturing/storage cells (%)	44.4	61.6	74.4	50.8
	P:M ratio	0.15	0.07	0.07	0.16
Dog 3-1	proliferative cells (%)	7.5	5.2	5	4.2
	maturing/storage cells (%)	43.5	53.4	59.2	51.8
	P:M ratio	0.17	0.1	0.08	0.08
Dog 3-2	proliferative cells (%)	5.4	5	5.8	4
	maturing/storage cells (%)	45	55	60	58
	P:M ratio	0.12	0.09	0.1	0.07

cyte function in healthy dogs (9).

In this study, leukocyte levels were higher in dogs that received injections every day than in the other groups. There was no significant difference between group 1 and group 2 in M:E ratio. We speculate that administration of G-CSF every other day sufficiently stimulates bone marrow granulocytes to change compartment, but not enough to cause large numbers of maturing cells to move from the bone marrow into the circulation pool. Based on these results, daily administration of G-CSF seems to accelerate its effects.

Based on comparison of M:E ratios, G-CSF effects on the bone marrow were considered to be sustained by repeated injection between day 0 and day 9. An increase in the myeloid compartment of bone marrow and relative erythroid hypoplasia contribute to M:E ratio increases (32). The effect of G-CSF was prolonged for some time after last administration, based on comparison of M:E ratio between day 0 and day 12-13. A previous study reported that the M:E ratio returned to pretreatment level 2 weeks after the last treatment (37), leading us to suggest that it will take several days for the effect of G-CSF on M:E ratio of bone marrow to decrease after treatment.

In the present study, an increase of P:M ratio in the first 3 days of daily rhG-CSF treatment demonstrates that daily G-CSF administration leads to expansion of progenitor cells rather than differentiation of proliferative cells into maturing cells. Dogs that received injections every two or three days did not demonstrate an increase in P:M radio, implying that these regimens are not sufficient to expand the granulocyte precursors. After day 3, P:M ratio did not significantly change in most of groups, indicating that both immature and mature neutrophil pools increased to a similar degree (32).

In a previous report, 5 μg/kg/day administration of rcG-CSF in normal dogs was well-tolerated, with marked leukocytosis being observed in response to the treatment (37). However, administration of 1 or 3 μg/kg/day of rhG-CSF to patients with idiopathic neutropenia showed a cyclic response because the maturation of progenitors responsive to G-CSF and the movement of mature neutrophils from bone marrow were faster than the repopulation of progenitor cells (12). Reported side effects related to administration of rhG-CSF in humans include medullary bone pain associated with fast release of mature cells from the bone marrow, aggravation of preexisting inflammatory conditions, systemic and local infection in response to injection, splenomegaly, thrombocytopenia, and alopecia (14). In animals, the most critical adverse effect is the development of antibodies against rhG-CSF, which neutralize endogenous G-CSF (8,17). However, such antibodies are likely to develop only after prolonged use (26) or high-dose treatment (19). Normal dogs treated with rhG-CSF at a dose of 10 μg/kg/day for 30 days (8) and a dog treated with rhGM-CSF at a dose of 150 μg/kg/day subcutaneously for 10-12 days developed antibodies (19). Although many previous studies in hamsters, primates, and cattle have shown that the level of leukocytosis is related to the dose of rhG-CSF (4,5,35,38), abrupt stimulation of bone marrow in response to high-dose G-CSF might require increased nutrients and lead to other side effects. Moreover, lower doses can also cause sufficient leukocytosis to combat infectious agents without developing adverse effects (37). In the present study, subcutaneous administration of 5 μg/kg/day rhG-CSF to normal dogs produced sufficient leukocytosis to combat infectious agents, and no side effects were observed.

There are many factors associated with granulopoiesis. IL-3 and G-CSF have been shown to interact to accelerate production of multipotential haematopoietic precursors (11). The interaction of other factors with G-CSF should be investigated for further understanding. The sample size of the current research was not sufficient to enable consideration of the effect of other factors or interactions between them; therefore, studies that are larger in scope are needed. In addition, it will be more meaningful to consider not only normal dogs, but also the dogs with myelosuppression. However, it is clear that administration of rhG-CSF is frequency-dependent and will be an effective treatment in dogs with neutropenia.

Conclusion

The present study demonstrates that peripheral and bone marrow response depends on the frequency of rhG-CSF administration in dogs. Furthermore, the effects of rhG-CSF administration on bone marrow were investigated based on short-term bone marrow aspiration. Overall, rhG-CSF was well-tolerated and showed no adverse effects. According to the present study, subcutaneous administration of rhG-CSF has cell-specific, frequency-dependent effects on bone marrow and peripheral blood, and the effects of rhG-CSF administration on bone marrow were sustained throughout the trial. Clinicians might be able to use the findings of this study to make suitable treatment regimens for patients with leukopenia.

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개에서 재조합 과립구 자극 인자 (rh G-CSF)의 투여 간격에 따른 말초혈액과 골수의 반응에 대한 연구

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요 약 : 본 연구는 사람 재조합과립구자극인자 (rhG-CSF) 투여 간격에 따른 말초혈액과 골수의 변화를 평가하였다. 사람 재조합과립구자극인자는 개에서 항암치료 후 나타나는 골수억압 등에서 유용한 치료효과가 있는 것으로 알려져 있으나 그 투여간격에 따른 연구는 아직 진행되지 않았다. 따라서 본 연구에서는 다음과 같은 간격으로 실험 기간 동안 각각 2마리씩 3그룹으로 체중당 5 μg으로 사람 재조합과립구자극인자를 주사하였다; (1) 하루에 한번 주사; 그룹 1, (2) 2일에 한번 주사; 그룹 2, (3) 3일에 한번 주사; 그룹 3, 말초혈액 분석과 100개의 직접 현미경 세포계수는 매일 실시하였다. 골수천자는 0일차, 3일차 9일차 마지막으로 말초혈액상 총 백혈구수가 정상범위내로 떨어지는 12일차 혹은 13일차에 시행하였다. 사람 재조합과립구자극인자는 모든 실험견에서 잘 투여되었으며 부작용을 보이지 않았다. 이러한 본 연구의 결과에 따르면 체중당 5 μg의 사람 재조합과립구자극인자의 투여는 말초혈액과 골수에서 세포특이적이고 투여간격에 대체적으로 비례하는 효과를 보였으며 사람 재조합과립구자극인자 투여가 반복될수록 그 효과는 증가하였으며 사람 재조합과립구자극인자 투여를 중지한 이후에도 골수에 미치는 효과는 3일이상 지속되는 것으로 보여졌다. 따라서 본 연구의 결과를 통해 임상가들은 적절한 치료 간격을 세울 수 있을 것으로 기대된다.

주요어 : 사람 재조합과립구자극인자, 개, 백혈구 감소증