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The Effect of Granulocyte Macrophage-Colony Stimulating Factor upon the Induction of Peripheral Blood Dendritic and Natural Killer Cells When Given Simultaneously with a Slow Continuous Doxorubicin Infusion

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Abstract

It has been demonstrated that it is safe to give Gm-Csf, together with Doxorubicin, by continuous intravenous infusion, thereby substantially increasing the amount of Doxorubicin administered to the average patient, and assuring that each patient receives an individually-determined safe and maximal amount of drug. It is known that Gm-Csf is a potent inducer of components that are major factors in an immunologic attack upon neoplasms. For that reason, we thought it would be worth evaluating in 4 patients' surface markers of dendritic precursor cells, dendritic cells [DC], and natural killer [NK] cells during the infusion. While there was substantial variation in individual responses, all 4 patients receiving Gm-Csf developed persistent marked increases in cells with each of these markers. The significance of these findings will be discussed.

Keywords

Doxorubicin Infusion Dendritic, Killer Cells

1. Introduction

The combination of anti-cancer chemotherapy with an immuno-therapeutic attack upon the neoplasm is an active area of study at the present time. We have studied the effect of giving Granulocyte Macrophage-Colony Stimulating Factor

[#]Dr. Stoloff is deceased.

[Gm-Csf] concurrently with Doxorubicin given by slow intravenous infusion. Doxorubicin did not prevent the stimulation of natural killer cell production and their precursors by Gm-Csf nor was any adverse effect upon the anti-tumor activity of Doxorubicin noted.

2. Materials and Methods

2.1. Patient Selection

Two of the patients had extensive metastatic carcinoma of the breast, one, 35 years old, primarily involving the liver and lung, and a few bony metastatic lesions, tumor stage M1; the other, 41 years old, had extensive hepatic and skin metastases, but no obvious bony metastases, either by bone scans or x ray, tumor stage M1. The third patient, a nine year old male, had a very large angiosarcoma, probably arising in the scapula, with no evidence of metastatic disease; tumor stage T3, N0, and M0. The fourth patient, a 22-year-old male, had a small cell anaplastic carcinoma, probably arising in the lung, with widespread pulmonary and hepatic metastases, and several small osteolytic lesions believed to be due to metastases; tumor stage M1. None of these patients had received prior anti-cancer chemotherapy, radiotherapy, or immunotherapy. Informed consent was obtained from all patients, and the protocols were approved by the pertinent institutional review boards.

2.2. Course of Therapy

Full details of the course received by these patients have been described in previous articles [1] [2]. All patients received 250 ucgm/M^2 of Gm-Csfsc, beginning 2 days before the Doxorubicin infusion was started, and continuing throughout therapy. Doxorubicin, 8 mgm/M^2 was given as a 24 hour infusion/ day. With all four patients, the peripheral W.B.C rapidly increased and the infusion was continued at this dose until the W.B.C fell to less than 7000/ul, or platelet count fell to less than 25,000/ul.

1) Assay of monocytes and dendritic cells precursors

Forty ml. of peripheral blood was drawn at each interval from patient #1, 80 ml. of blood was drawn at each interval from patients 2, 3, and 4. Buffy coats were separated by Ficoll gradient ultracentrifugation and cell populations assayed using a B.D. flow cytometer. The following cell markers, believed to represent factors related to anti-tumor immunotherapy, were evaluated, CD1c [3] [4] [5] [6], as a dendritic cell marker, CD34 [7] [8] as a dendritic precursor cell marker, and CD56 [9] [10] as a natural killer cell marker. Fluorescent cell surface markers were obtained either from Becton Dickinson or from other sources selected by them. Peripheral cell counts were determined with a Coulter counter. Number of cells in the peripheral blood with monocytoid features was determined by a trained cytologist using H and E and/or geimsa stains Studies of patient's 2, 3, and 4 were performed as soon as blood was drawn, blood from patient #1 was drawn, packed in ice, and processed 3 to 6 hours later.

2) Results of this study are presented in Table 1.

It is recognized that the labels chosen for each of the groups are arbitrary and that multiple markers and that several sub-groups for each of these groups exist. It is believed that the markers chosen would best represent the chosen group. Despite the small sample size, it is our belief that the results indicate that the simultaneous administration of a potent anti-tumor chemo-therapeutic agent and an agent that induces an immunologic attack upon a tumor is feasible and may act synergistically against the neoplasm.

3. Discussion

The paths to anti-cancer immunotherapy are complex and our knowledge of its workings is incomplete. We do know, however that the mobilization of dendritic precursor cells, mature dendritic cells, and native killer cells are essential

Table 1. Effect of Gm-Csf on killer cells and their precursors when given a continuous infusion of Doxorubicin.

	Pt #	Pre Rx	1 - 2 Days of	5 - 8 Days of	1 - 2 Days Post	Total Dose
		Value	Infusion	Infusion	Infusion	Infusion M^2
WBC/ct/ul	1	4850	16,585	22,915	2150	104
	2	6635	14,560	19,900	1800	128
	3	14,330	21,000	29,900	1835	72
	4	5130	13,355	14,270	950	128
Monocyte	1	385	2955	4490	110	
Ct/ul	2	710	4400	7000	465	
	3	1600	11,350	12,050	910	
	4	370	1980	7750	330	
% Immature	1	0	35	55	74	
Monocytes	2	0	50	70	62	
	3	6	49	55	87	
	4	2	22	46	88	
Total CD34	1	4	334	773	43	
Cells/ul	2	1	189	247	34	
	3	11	606	677	177	
	4	2	805	301	96	
Total Cd1c	1	107	222	787	44	
Cells/ul	2	84	589	623	21	
	3	222	892	1409	16	
	4	75	103	982	37	
TotalCD56	1	98	231	782	333	
Cells/ul	2	22	44	288	106	
	3	178	589	540	277	
	4	49	379	811	87	

components in one of these paths [11]. It is also recognized that Gm-Csf is a potent inducer of this process. We therefore thought it would be of value to determine whether Doxorubicin, given by slow intravenous infusion, would interfere with the effect of Gm-Csf on this pathway. The results presented in table one; however, indicate that Gm-Csf is capable of initiating a major immuno-stimulating pathway despite the presence of a significant blood level of Doxorubicin.

Until recently the simultaneous use of hematologic growth factors such as G-Csf and Gm-Csf with anti-cancer agents has been discouraged, primarily because of the marked increase in toxicity when either has been given with either 5-fluorouracil or topotecan [12] [13]. Recent studies however have demonstrated that these agents can be used safely with several anti-neoplastic drugs such as Doxorubicin, Ifosphamide, or vincristine [2] [3] [14] [15] [16] [17]. We have also found that 5-fluorouracil and F.U.D.R. can be given safely, provided that the dose of the fluorinated pyrimidine is substantially reduced. We have noticed significant anti-tumor activity when F.U.D.R. is given intra-arterially and the dose is reduced to 20% to 30% of the standard dose. [1] [14] [15].

The disadvantages of this protocol have been noted in a previous publication [1] [2], and primarily relate to the complexity of the procedure. Another possible disadvantage is that the slow infusion leads to the absence of high peak levels of Doxorubicin. This may allow neoplasms with an effective Doxorubicin efflux pump to survive [1] [2]. The fact that multiple studies have shown that the anti-tumor effect of Doxorubicin against many tumor types is independent of type of schedule negates this problem [18] [19] [20] [21]. The third possibility is that Gm-Csf stimulates the growth of the tumor or protects the tumor against the chemotherapeutic agent [22] [23]. Although this possibility has been raised, especially concerning hematologic neoplasms, we know of no reports that suggest that this is true. The possibility that the use of Gm-Csf increases the risk of cardiac toxicity has been shown to be untrue in this scenario since it has been noted that a large number of patients receiving between 600 and 1400 mgm/M^2 of Doxorubicin using this protocol failed to show any sign of cardiomyopathy [1] [2]. However, this possibility must be considered when other combinations of biologic anti-tumor agents and agents such as Doxorubicin are considered. For example, it is known that the concurrent use of trastuzumab and Doxorubicin significantly increases the occurrence of cardiotoxicity. [24]. Several other tyrosine kinase inhibitors have a similar effect [25].

Advantages

1) The simultaneous of Gm-Csf with a specific anticancer agent such as Doxorubicin significantly increases the amount of Doxorubicin tolerated by the average patient [1] [2].

2) It permits the total dose of Doxorubicin [and other anti-tumor agents] [1] [2] received by the patient to be individually determined and not determined by a set protocol dose. The variation in dosage tolerated by various patients is a major impediment to using standard dosage schedules.

3) Theoretically the simultaneous use of an immunologic attack with anti-cancer chemotherapy might act in a synergist manner against the neoplasm. Thus, fragments of dying neoplastic cells, providing dendritic cells with tumor specific antigens, are being presented in large numbers to immunologic competent cells such as NK and dendritic cells.

4) There is evidence that Gm-Csf may act independently as an anti-tumor agent against several types of neoplasms [26] [27].

It was not possible to directly determine the anti-tumor effect of the single course of Gm-Csf and Doxorubicin in any of the 4 patients for several reasons. All patients received further anti-cancer therapy and the interval between courses was too short to expect radiographic evidence of an anti-tumor effect, as all patients received further aggressive chemotherapy as soon as their hematologic parameters returned to normal [1] [2]. Subjectively, the patient with the angiosarcoma reported that his pain, which, before therapy had been severe, had almost totally disappeared. The breast cancer patient with bony metastases also reported marked pain relief as did the patient with the anaplastic carcinoma. This finding is commonly recognized as an indication of tumor necrosis. We have reported that repeated courses of infusional Gm-Csf with Doxorubicin, as well as similar courses utilizing Ifosphamide, or Vinorelbine have been very effective in tumor control [1] [2].

The dose of GM-Csf was arbitrarily chosen and may have been sub-optimal. It has been demonstrated that a much larger dose of Gm-Csf [500 ugm/M^2] when given daily is well tolerated and is associated with a substantially greater increase in the number of leukocytes as compared to the 250 ugm daily dose [28]. This suggests that if we raise the dose to Gm-Csf the immune mediated antitumor effect may be enhanced. It is also been shown that other agents such as IL4, IL12, or IL21 when given with GM-Csf influences the effectiveness of the immunologic antitumor response [29] [30] [31]. This suggests that combining Gm-Csf with other immunologic agents may enhance the effect that we have seen. It is also possible that other cytokine would be more effective than Gm-Csf as an immunologic stimulant. This may be worth studying.

4. Conclusions

Four patients received Gm-Csf 250 ugm/M^2 s.c. daily together with Doxorubicin 8 mgm/M^2 given by continuous infusion until hematologic toxicity occurred. Cells believed to be involved in the anti-tumor immune responses were monitored using cell surface markers. These included CD34 cells as a tracer for DC progenitor cells, CD1c for dendritic cells, and CD56, as a tracer for Natural Killer cells. All these cells showed a rapid and marked rise. Thus, the number of CD34-labelled cells rose from an average of 4.5/ul to an average of 497 cells/ul, the number of CD1c labelled cells from 122/ul to 950/ul, and CD56 labelled cells from 88/ul to 605/ul. Mature monocyte counts markedly increased and immature monocytes and other very primitive nucleated cells, extremely rare before therapy, appeared in large numbers. These immature cells disappeared once the

Gm-Csf was stopped. The average tolerated dose of Doxorubicin was 108 mgm/M^2.

The destruction of neoplastic cells with the ejection of tumor fragments into the peripheral blood in the presence of large number of dendritic and natural killer cells, therefore, may yield a substantial increase in the anti-tumor effectiveness of Doxorubicin and increase the potency of the body's anti-tumor immunologic defense mechanisms.

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