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Research paper

Mechanism of transient dyspnea induced by pegylated-liposomal doxorubicin (Doxil™)

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While mucositis and hand–foot syndrome are the main limiting toxicities of pegylated-liposomal doxorubicin, a small proportion of patients develop transient dyspnea at the initiation of drug infusion. Of the first 35 patients in a phase II study of pegylated-liposomal doxorubicin, three developed dyspnea, two low back pain and two pain at the site of tumor, within 1–5 min after starting the pegylated-liposomal doxorubicin infusion. The symptoms resolved within 5–15 min of stopping the infusion. In each case, the infusion was restarted without adverse effect. The mechanism of these symptoms is unclear. Because the dyspnea was reminiscent of that seen with hemodialysis neutropenia, complete blood counts were obtained in four of these patients approximately 2 min after the onset of symptoms. In all four patients, relative neutropenia was present (ANC 35, 3, 24 and 46% of pretreatment) that resolved by the end of the pegylated-liposomal doxorubicin infusion. Pegylated-liposomal doxorubicin stimulated neutrophil adhesion to human umbilical vein endothelial cells *in vitro* at concentrations predicted to be present in plasma during the initiation of treatment. Thus, pegylated-liposomal doxorubicin can induce an increase in neutrophil adhesion directly. We conclude that one mechanism of pegylated-liposomal doxorubicin-induced acute dyspnea is a transient sequestration of neutrophils in the pulmonary circulation, resulting in a decrease in compliance and associated dyspnea. In the patients in this study, these symptoms were transient, mild and not life threatening. Pegylated-liposomal doxorubicin is generally well tolerated and we do not routinely use premedications in patients receiving pegylated-liposomal doxorubicin. [© **1998 Rapid Science Ltd.**]

Key words: Cancer, chemotherapy, liposome, lung, neutrophil.

Introduction

There is interest in administering chemotherapy drugs in liposomes as a means of altering the therapeutic index of the drug. Liposomal doxorubicin is of particular interest since there may be less cardiotoxicity, presumably because there is no high concentration of free drug after i.v. administration. Furthermore, liposomal doxorubicin may have greater anti-tumor efficacy, presumably due to increased delivery to the tumor via leakage from the abnormal tumor microvasculature, and/or some degree of prolonged drug release similar to continuous infusion chemotherapy. Pegylated-liposomal doxorubicin (Doxil™) is a unique form of liposomal doxorubicin in which the doxorubicin is contained in liposomes that are coated with polyethylene glycol. The polyethylene glycol confers useful properties including a diminished uptake by the reticuloendothelial system, leading to a much longer half-life in blood (about 50–60 h) and a different toxicity profile than non-pegylated liposomes.[1–3] Animal studies have shown that pegylated liposomes localize to implanted tumors[4] and deliver more doxorubicin to the tumor than free doxorubicin.[2,5] In patients with AIDS and Kaposi sarcoma, pegylated-liposomal doxorubicin administration has also been found to deliver more doxorubicin to the Kaposi sarcoma lesions than does administration of free doxorubicin; pegylated-liposomal doxorubicin treatment also delivers more doxorubicin to the Kaposi sarcoma lesion than to normal adjacent skin.[6] Pegylated-liposomal doxorubicin is highly effective for Kaposi sarcoma in patients with AIDS and has only minor toxicity.[7] Preliminary evidence suggests activity in other tumors as well, including breast, ovarian, and

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head and neck cancer.[8] In general, the toxicity profile of pegylated-liposomal doxorubicin is more similar to that of doxorubicin given by continuous infusion.[7,8] In contrast, the toxicity profile of non-pegylated liposomal daunorubicin more closely resembles that of doxorubicin given by bolus infusion.[9] Pegylated-liposomal doxorubicin causes less cardiotoxicity than the same dose of free doxorubicin in animals,[10,11] and nausea, vomiting and alopecia are much less common with pegylated-liposomal doxorubicin than with free doxorubicin.[7] In addition, evidence suggests that inadvertent extravasation of pegylated-liposomal doxorubicin causes only mild transient irritation,[12] in contrast to free doxorubicin. A self-limited infusion reaction consisting of dyspnea, back pain or flushing has been reported in a small percentage of patients receiving pegylated-liposomal doxorubicin.[7]

In the course of phase II trials of pegylated-liposomal doxorubicin in solid tumors, we observed three patients who developed transient shortness of breath within minutes of the initiation of infusion of pegylated-liposomal doxorubicin. Two patients with intra-abdominal tumors developed transient low back pain with a similar time course. The etiology of this reaction is unknown, but the symptoms are reminiscent of those seen with hemodialysis neutropenia, which occurs when cuprophane dialyzer membranes are used. Early studies revealed that transient marked neutropenia resulting from reversible pulmonary leukostasis occurred in all patients during the initiation of hemodialysis with cuprophane membranes.[13,14] This leukostasis was shown to be due to an increase in granulocyte adhesiveness mediated by C5a, resulting in both increased neutrophil adhesion to endothelial cells and the formation of homotypic aggregates of granulocytes with embolization and trapping of these aggregates in the microcirculation.[15-17] This pulmonary sequestration of neutrophils can result in lung dysfunction, with mismatching of ventilation-perfusion, pulmonary edema and shortness of breath.[18] The transient nature of hemodialysis neutropenia has been found to be due to a selective desensitization of neutrophils to stimulation by C5a, in the face of continued production of C5a by interaction of plasma with the dialyzer membrane.[19]

Because of the clinical similarities between the observed syndrome following administration of pegylated-liposomal doxorubicin and the phenomenon of hemodialysis neutropenia, we performed studies to examine the possibility that pulmonary leukostasis contributes to the symptoms seen with the initiation of pegylated-liposomal doxorubicin therapy. We examined the circulating blood counts of four patients who experienced shortness of breath or back/tumor pain at the initiation of i.v. pegylated-liposomal doxorubicin, as well as two patients who had no infusion reaction. In addition, the effect of pegylated-liposomal doxorubicin on neutrophil adhesion to human umbilical vein endothelial cell (HUVEC) monolayers was examined. Transient peripheral neutropenia was observed during symptoms in each patient who experienced shortness of breath or back/abdominal pain; in each case, this neutropenia reversed by the end of the pegylated-liposomal doxorubicin infusion. Pegylated-liposomal doxorubicin was also found to augment neutrophil adhesion to HUVECs *in vitro*. The data suggest that the development of transient shortness of breath or back/abdominal pain that occurs in a small proportion of patients at the start of their first course of pegylated-liposomal doxorubicin is due to transient neutrophil sequestration in the pulmonary circulation and/or the tumor microvasculature.

Materials and methods

Pegylated-liposomal doxorubicin administration

Pegylated-liposomal doxorubicin (Doxil™; Sequus Pharmaceuticals, Menlo Park, CA) was administered at 55 mg/m^2 in about 500 ml D5W by i.v. infusion over 2-4 h at a rate less than 1 mg/min. Treatment was repeated at intervals of 28 days or more based on toxicity. The study was approved by the University of Minnesota Institutional Review Board and informed consent was obtained from all patients.

Preparation of peripheral blood cells

Neutrophils were prepared from heparinized (2 units/ml) human venous blood as described.[20]

Fluorescence labeling of cells

Neutrophils were labeled with calcein AM (Molecular Probes, Eugene, OR)[21] by incubating 5×10^6/ml cells with 50 μg of calcein AM for 30 min at 37°C in 18 ml of calcein-labeling buffer (HBSS without Ca or Mg containing 0.02% BSA). Cells were then washed twice with calcein-labeling buffer and resuspended in the desired media.

Endothelial cell adhesion assay

The adhesion of neutrophils to HUVEC monolayers was determined as previously described.[22,23] Briefly, HUVECs (Clonetics, San Diego, CA) were passaged 1:5 in T-25 flasks (Costar) no more than three times before plating in 96-well microtiter plates at 3000 cells/well. HUVECs were grown to confluence in 96-well microtiter plates in EGM media (Clonetics) and fed every 24 h. Using the adhesion assay described below, no difference in neutrophil adhesion was observed and, as expected, no difference in surface expression of CD54 (ICAM-1) or CD62E (E selectin, ELAM-1) was noted using HUVECs passaged once compared with those passaged five times. In some experiments, the HUVECs were stimulated by culture for 4 h with 50 ng/ml tumor necrosis factor (TNF)-α (Cetus, Emeryville, CA). The wells were then washed four times with adhesion buffer (DMEM+5% HIFBS) and 25 μl of adhesion buffer containing the indicated concentration of pegylated-liposomal doxorubicin was added to each well. Then, 100 μl of adhesion buffer containing 10^5 calcein-labeled neutrophils was then added and the plates were incubated at 37°C in 5% CO_2 for 30 min. The wells were then aspirated and washed four times with endo wash buffer (HBSS+4% HIFBS), and the fluorescence was quantitated with a Millipore fluorescence plate reader using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Control standard curves demonstrated that pegylated-liposomal doxorubicin did not interfere with the fluorescence assay. For each condition, quadruplicate wells were tested and values are reported as the mean ± SD. Each experiment was performed at least four times using different HUVEC subcultures.

Statistical analysis

Effects of pegylated-liposomal doxorubicin on neutrophil adhesion to HUVECs was analyzed by the Mann-Whitney *U*-test when appropriate.

Results

In the course of a phase II trial of pegylated-liposomal doxorubicin, transient shortness of breath occurred in two patients and low back pain in one patient within minutes of beginning the infusion. In all three patients the symptoms resolved quickly. Because this syndrome was reminiscent of hemodialysis neutropenia, complete blood counts were performed in four subsequent patients who developed similar symptoms and two patients who developed no symptoms during pegylated-liposomal doxorubicin infusion.

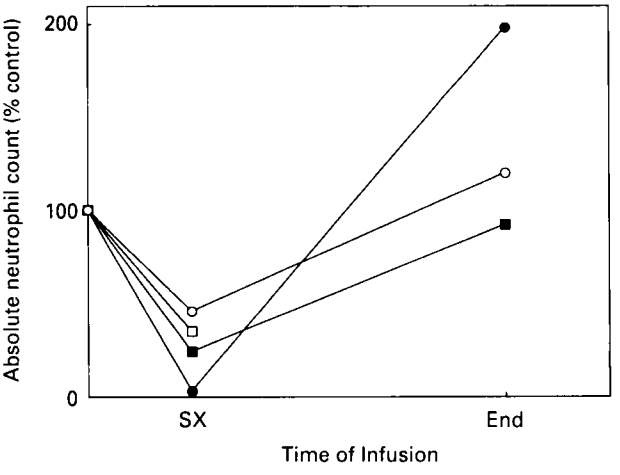

Figure 1. Absolute neutrophil counts (as a percent of pretreatment values) before treatment (time=0), at the time of symptoms as described in the results (SX) and at the end of the pegylated-liposomal doxorubicin infusion (End) for patients 4 (open squares), 5 (solid circles) 6 (solid squares) and 7 (open circles) as described in the text.

A fall in circulating neutrophil count was observed in each of the four patients who developed shortness of breath or back/abdominal pain shortly after the initiation of pegylated-liposomal doxorubicin, in whom blood counts were examined (see below and Figure 1). No change in circulating neutrophil count at 10 min after starting pegylated-liposomal doxorubicin or at the end of the infusion was observed in two patients who experienced no symptoms during the pegylated-liposomal doxorubicin infusion (not shown).

Of 35 patients treated with pegylated-liposomal doxorubicin, shortness of breath or low back or abdominal pain developed in seven within 10 min of starting the infusion of their first treatment. One of the seven patients who developed this syndrome had been pretreated with diphenhydramine and cimetidine, while the other six patients had received no premedications. The first four patients who developed this syndrome with the first treatment were premedicated with diphenhydramine, cimetidine and hydrocortisone for subsequent treatments. One patient who received no premedication and developed this syndrome with his first treatment, received subsequent treatments with no premedication and no recurrence of the syndrome. In the seven patients who developed this syndrome with their first treatment, 15 subsequent courses of

treatment were given with no recurrence of the syndrome. Of the 28 patients who experienced no reaction to their first treatment, only one received premedication with diphenhydramine and cimetidine. No patient experienced this symptom complex with subsequent treatments. In the 28 patients who did not experience this syndrome with their first treatment, a total of 96 courses have been given without appearance of this syndrome.

Clinical course of patients experiencing shortness of breath or pain at the onset of pegylated-liposomal doxorubicin infusion

Patient 1. A 39-year-old white woman with abdominal leiomyosarcoma developed shortness of breath 1–2 min after the pegylated-liposomal doxorubicin infusion was started. Examination of the chest was normal and the blood pressure was 160/100. She was given 50 mg diphenhydramine and the symptoms resolved within 5 min of onset. She was given 300 mg cimetidine and 10 mg dexamethasone over 15 min, and 30 min after beginning the infusion the infusion was restarted with no recurrence of symptoms. She received 50 mg diphenhydramine, 100 mg hydrocortisone and 300 mg cimetidine as premedication for each of her subsequent five treatments.

Patient 2. A 70-year-old white woman with abdominal leiomyosarcoma developed low back pain about 5 min after starting the pegylated-liposomal doxorubicin infusion. The infusion was stopped and she was given 50 mg diphenhydramine, and the symptoms resolved within another 5–10 min. She was given 100 mg hydrocortisone and 300 mg cimetidine over the next 15 min, and the infusion was restarted without recurrence of symptoms. She received 50 mg diphenhydramine, 100 mg hydrocortisone and 300 mg cimetidine as premedication for each of her subsequent five treatments.

Patient 3. A 73-year-old white man with pulmonary metastases of leiomyosarcoma developed shortness of breath about 2 min after starting the pegylated-liposomal doxorubicin infusion. The infusion was stopped and the shortness of breath resolved within 5 min. He was given 50 mg diphenhydramine and 300 mg cimetidine, and 20 min later the infusion was restarted without recurrence of symptoms. He received 50 mg diphenhydramine and 100 mg hydrocortisone as premedication for his subsequent treatment.

Patient 4. A 37-year-old white man with massive metastatic involvement of the liver with a low grade leiomyosarcoma of gastrointestinal origin developed abdominal pain about 5 min after starting the pegylated-liposomal doxorubicin infusion. The infusion was stopped and the pain improved within 2 min, at which time a complete blood count was obtained (see Figure 1). He was given 50 mg diphenhydramine and 300 mg cimetidine, and 10 min later the infusion was restarted without recurrence of symptoms. He received 50 mg diphenhydramine and 100 mg hydrocortisone as premedication for his subsequent treatment.

Patient 5. A 38-year-old white man with leiomyosarcoma of gastrointestinal origin metastatic to the liver developed shortness of breath about 2–5 min after starting the pegylated-liposomal doxorubicin infusion. The infusion was stopped and the shortness of breath improved within about 5 min. A complete blood count was obtained shortly after the onset of symptoms (see Figure 1). Approximately 10 min later, the infusion was restarted without recurrence of symptoms. He received four subsequent treatments with no premedication and no recurrence of symptoms.

Patient 6. A 55-year-old white woman with widely metastatic leiomyosarcoma with massive liver involvement developed low back and abdominal pain about 3 min after starting the pegylated-liposomal doxorubicin infusion. The infusion was stopped and the pain improved within 2 min, at which time a complete blood count was obtained (see Figure 1). Some nausea was present about 10 min later and she was given 1 mg of granisetron i.v. The pain had completely resolved 25 min after starting the infusion and 10 min later the infusion was restarted with no recurrence of symptoms.

Patient 7. A 34-year-old white man with epithelioid sarcoma metastatic to lung and pleura developed shooting pain/tingling sensation in the low back lasting several seconds about 2–3 min after starting the pegylated-liposomal doxorubicin infusion. The sensation recurred intermittently over about 1 min, and the infusion was stopped and the sensation resolved within 2 min, at which time a complete blood count was obtained (see Figure 1). Ten minutes later the infusion was restarted with no recurrence of symptoms.

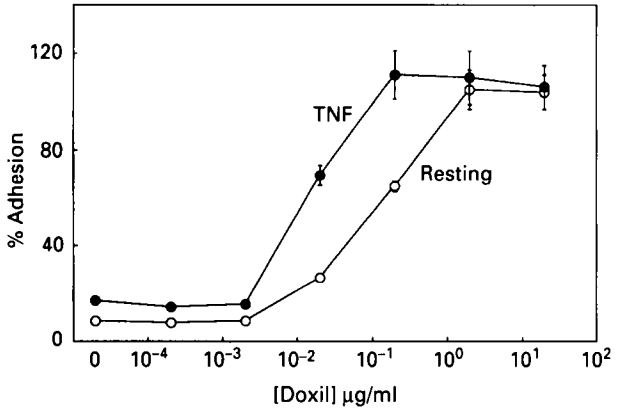

Figure 2. Effects of pegylated-liposomal doxorubicin on neutrophil adhesion to HUVECs. HUVECs were grown to confluence in 96-well microtiter plates, and used directly (open circles) or stimulated by adding 50 ng/ml TNF-α and incubating 4 h at 37°C (closed circles). The wells were then washed, pegylated-liposomal doxorubicin at the indicated final concentration was added and 100 μl of adhesion media containing 10^5 neutrophils was added. The plates were incubated at 37°C for 30 min in 5% CO_2, and the wells were then washed and the number of adherent neutrophils determined with a fluorescence plate reader. Values are shown as the percent of added neutrophils remaining adherent to the HUVEC monolayers and represent the means ± SD of four separate experiments.

Effects of pegylated-liposomal doxorubicin on neutrophil adhesion to endothelial cells

Pegylated-liposomal doxorubicin was tested for its ability to alter neutrophil adhesion to HUVECs. When neutrophils were incubated for 30 min with a monolayer of resting (Figure 2, open circles) HUVECs in the presence of media containing buffer (D5W) and washed as described in the Methods, few neutrophils remained adherent to the HUVECs. When this assay was performed in the presence of various concentrations of pegylated-liposomal doxorubicin, a dose-dependent increase in neutrophil adhesion was observed (Figure 2). An increase in neutrophil adhesion to HUVECs occurred at lower concentrations of pegylated-liposomal doxorubicin, when HUVECs were activated by stimulating for 4 h with 50 ng/ml TNF-α, an inflammatory cytokine (Figure 2, closed circles).

Discussion

Encapsulation of doxorubicin in pegylated liposomes enhances its efficacy and favorably alters its toxicity profile.[7] Pegylated-liposomal doxorubicin has been found to have high efficacy and minimal toxicity in the treatment of Kaposi sarcoma in patients with AIDS.[7] In addition, phase I studies have suggested that pegylated-liposomal doxorubicin may have efficacy in a variety of other malignant diseases as well, including breast, ovarian, and head and neck cancer.[8] Pegylated-liposomal doxorubicin is simple to administer and is generally well tolerated, with the most common dose-limiting toxocities being mucositis, hand-foot syndrome and myelosuppression.[7,8]

In the course of phase II studies of pegylated-liposomal doxorubicin given at a dose of 55 mg/m^2 every 28 days, we observed a low incidence of transient shortness of breath, back pain or pain in the region of a large tumor burden shortly after beginning the pegylated-liposomal doxorubicin infusion. Our results demonstrate that pegylated-liposomal doxorubicin can increase the adhesion of neutrophils to HUVEC monolayers and that relative neutropenia occurs in patients experiencing an infusion reaction. The data suggest that transient sequestration of circulating neutrophils in the pulmonary circulation contributes to the dyspnea, while transient sequestration in the tumor microvasculature may contribute to pain in regions bearing large tumor burdens. Why this syndrome only occurs in a small percentage of patients is unknown; however, in the two patients studied who did not experience an infusion reaction, no change in neutrophil count was observed.

The frequency of recurrence of this syndrome in patients receiving monthly pegylated-liposomal doxorubicin treatments appears to be low as none of the patients in this study experienced a recurrence. However, the incidence of the syndrome in this study in patients with solid tumors receiving their first treatment using 55 mg/m^2 of pegylated-liposomal doxorubicin appears to be higher than that seen using 20 mg/m^2 in patients with AIDS and Kaposi sarcoma.[7] Four of 16 patients with solid tumors receiving rapid infusions of 25-50 mg/m^2 pegylated-liposomal doxorubicin experienced a similar infusion reaction.[3]

The efficacy of premedication with diphenhydramine, cimetidine and hydrocortisone is unproven. Indeed, in two patients who did not receive premedication this syndrome resolved without additional medication and the one patient who received further treatments did not experience a reaction in any of four subsequent treatments without premedication. In addition, this syndrome occurred in one patient who had received premedication. Finally, earlier studies demonstrated that very high doses of corticosteroids (estimated peak plasma levels of about 1 mg/ml) blunted, but did not completely prevent, neutropenia in an animal model mimicking hemodialysis neutropenia (D Hammerschmidt, personal communication). We

no longer use premedications in patients receiving pegylated-liposomal doxorubicin, thus simplifying treatment and minimizing additional side effects of premedications.

Conclusion

We conclude that one mechanism of pegylated-liposomal doxorubicin-induced acute dyspnea is a transient sequestration of neutrophils in the pulmonary circulation, resulting in a decrease in compliance and associated dyspnea. In the patients in this study, these symptoms were transient, mild and not life threatening. Pegylated-liposomal doxorubicin is generally well tolerated and we do not routinely use premedications in patients receiving pegylated-liposomal doxorubicin.

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