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Pulmonary epithelial permeability in patients treated with bleomycin containing chemotherapy detected by technetium-99m diethylene triamine penta-acetic acid aerosol (^{99m}Tc-DTPA) scintigraphy

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Purpose: To evaluate pulmonary epithelial permeability using ^{99m}Tc-DTPA scintigraphy in patients treated with bleomycin-containing regimens. ***Material and Methods:*** Twelve non-smoking chemotherapy-naïve patients with no clinical or radiological evidence of pulmonary disease and treated with bleomycin-containing chemotherapy were tested with ^{99m}Tc-DTPA scintigraphy before the first cycle and every 3 weeks until the third month after the end of chemotherapy (total cumulative dose of bleomycin 347.9 mg). ***Results:*** Pretreatment values ($T_{½}$ 74.93 minutes) of ^{99m}Tc-DTPA scintigraphy were significantly higher than those obtained after the total dose of bleomycin ($T_{½}$ 51.00 minutes) ($p < 0.001$). This difference was more important in the later evaluations especially, on the third week and third month measures after discontinuing treatment ($p < 0.001$). All the tests of Within-Subjects Effects were significant ($p < 0.001$). Comparing pretreatment and post-treatment scintigraphies the mean $T_{½}$ ^{99m}Tc-DTPA values decreased as the bleomycin dose increased. ***Conclusion:*** We conclude that cumulative bleomycin doses are related to increased pulmonary epithelial permeability at a dose of 256.5 mg. However, whether this is related to clinical toxicity is uncertain and large, multi-center prospective studies are needed.

Key words: pulmonary epithelial permeability, bleomycin, lung toxicity, ^{99m}Tc-DTPA scintigraphy

INTRODUCTION

BLEOMYCIN is an antitumoral and antibiotic drug produced by *Streptomyces verticillus*.[1] It is able to break the DNA double helix by producing free radicals, which is an oxygen and iron dependent process.[1,2]

The most important adverse effect of bleomycin is pulmonary toxicity, which may occur in 2 to 46% of patients treated with bleomycin-containing chemotherapy,[2–4] and can be fatal in 1 to 2% of these patients.[2,5,6] This toxicity was first described in 1972.[7] The predictor factors for pulmonary toxicity are: being over 70 years of age, cumulative bleomycin dose of more than 450 mg, concomitant pulmonary irradiation, renal failure, administration route, oxygen exposure,[7] smoke,[8] Granulocyte-Colony Stimulating Factor (GCSF) administration[9] and use of multiple antineoplastic agents.[7] The main symptoms are cough, dyspnea and fever.[10] The most common clinical signs are bilateral rales, tachypnea and cyanosis.[7]

Until now, there has been no consensus on when bleomycin administration should be stopped. Sleijfer et al. recommend that its administration be stopped when Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) decreases 60% from the basal value, or when this value decreases to less than 40%.[3] Comis et al. and Baehner et al. agree in stopping the prescription of bleomycin after DLCO decreases to less than 40%.[10,11] Weiss et al. believe that this medication should be stopped

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after DLCO decreases 20% from basal value.[12] Vital Capacity and Total Lung Capacity decrease during treatment with bleomycin, and should be correlated with the clinical signs.[13]

There are many methods to evaluate the pulmonary epithelial membrane in animal models. In 1977 Rinderknecht et al. showed that technetium-99m diethylene triamine penta-acetic acid aerosol (^{99m}Tc-DTPA) scintigraphy is a useful, sensitive and cheap method for evaluating the pulmonary epithelial membrane.[14] Taplin and Efros evaluated patients with interstitial pulmonary disease and reported that the clearance of ^{99m}Tc-DTPA scintigraphy is altered, reflecting impairment in the permeability of the alveolocapillary barrier.[15] The main aim of this study is to evaluate the pulmonary epithelial permeability using ^{99m}Tc-DTPA in patients with germ cell tumor or Hodgkin's disease, treated by bleomycin-containing regimens.

MATERIAL AND METHODS

Study population

From April 1999 to February 2002, 12 patients (10 males and 2 females) with testis or ovary germ cell tumor and Hodgkin's disease, were treated with bleomycin containing chemotherapy. None of these patients had any other disease or were smokers. They had no evidence of pulmonary disease or pulmonary symptoms 30 days before enrollment, nor had they received prior antineoplastic treatment or thoracic radiation. Patients had Eastern Cooperative Oncology Group performance status of 0 to 2 and had given written informed consent. The mean age of the patients was 28.6 years (range 21–43). These patients were treated at Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. The Ethical Committee had approved this protocol study.

Chemotherapy

Chemotherapy was given according to the BEP and ABVD protocols. In BEP protocol, cisplatin was given at a dosage of 20 mg/m^2 body surface area (BSA) over a period of 60 minutes by intravenous infusion on 5 consecutive days (days 1–5), in a 3 week-cycle. Etoposide was given at a dosage of 100 mg/m^2 BSA over a period of 60 minutes by intravenous infusion after cisplatin (days 1–5). Bleomycin was given at a dosage of 30 mg (unit) as an intravenous bolus on days 2, 9 and 16, during 2–4 cycles up to a cumulative bleomycin dose of 360 mg. In ABVD protocol, doxorubicin was given at a dosage of 25 mg/m^2 BSA as intravenous bolus on days 1 and 15. Bleomycin was given at a dosage of 10 mg/m^2 BSA as intravenous bolus on days 1 and 15. Vinblastin was given at a dosage of 6 mg/m^2 BSA as intravenous bolus on days 1 and 15, and dacarbazine was given at a dosage of 375 mg/m^2 BSA as intravenous bolus on days 1 and 15. The ABVD protocol was given every 4 weeks during 6 months, up to a cumulative bleomycin dose of 215.2 mg.

Table 1 Patient characteristics

Patient number	Age	Gender	Diagnosis (site)	Total bleomycin dose (mg)
1	43	M	Testis	360
2	30	M	Testis	360
3	24	M	Testis	270
4	31	M	Testis	360
5	25	M	Testis	270
6	23	M	Testis	360
7	28	M	Testis	360
8	41	M	Testis	360
9	25	F	Ovary germ cell	360
10	28	M	Testis	360
11	25	M	Testis	270
12	21	F	Hodgkin's disease	215.2

F: female M: male

Table 2 Values of T½ ^{99m}Tc-DTPA scintigraphy

Patient		Before CT	After C1	After C2	After C3	3 weeks later	3 months later
1	RL	75	78	76	82	53	42
	LL	132	103	76	86	57	36
2	RL	53	73	ND	43	50.5	27
	LL	56	77	ND	45	49	32
3	RL	91	73	94	NN	43	53
	LL	92	84	93	NN	41	50
4	RL	63	79	61	71	45	62
	LL	82	98	73	82	49	76
5	RL	45	44	74	NN	59	ND
	LL	49	48	91	NN	62	ND
6	RL	66	62	29	44	49	24
	LL	57	72	37	56	53	27
7	RL	63	47	50	69	55	53
	LL	63	50	52	83	63	52
8	RL	37	45	49	50	54	58
	LL	92	80	93	93	75	66
9	RL	68	66	70	64	47	64
	LL	75	64	67	60	56	64
10	RL	88	99	73	68	64	53
	LL	84	92	66	60	65	55
11	RL	84	49	48	NN	27	54
	LL	93	51	53	NN	26	64
12	RL	98	61	104	72	38	51
	LL	93	65	125	67	50	59

CT: chemotherapy; C1: first cycle; C2: second cycle; C3: third cycle; RL: right lung; LL: left lung; ND: not done; NN: not necessary

Aerosol lung scintigraphy

^{99m}Tc-DTPA was performed in all patients before chemotherapy, before every new cycle, 3 weeks after stopping treatment and 3 months later. ^{99m}Tc-DTPA was chelated by adding ^{99m}Tc-pertechnetate (^{99m}Tc-O_4^- IPEN-TEC,

Table 3 Mean value of T½ ^{99m}Tc-DTPA scintigraphy

^{99m}Tc-DTPA (time)	Mean bleomycin dose	Mean T½ ^{99m}Tc-DTPA	Standard Deviation	95% Confidence Interval	
				Lower limit	Upper limit
Before CT	0 mg	74.93	21.23	65.96	83.89
After C1	85.5 mg	69.14	17.96	61.56	76.73
After C2	171 mg	70.62	22.03	61.32	79.93
After C3	256.5 mg	**66.40** [a]	13.01	60.90	71.89
3 weeks later	347.9 mg	**51.27**[b]	11.34	46.48	56.06
3 months later	347.9 mg	**51.00**[b]	13.47	45.30	56.69

[a] $p = 0.003$ [b] $p < 0.001$ (Multivariate tests: Pillai's trace, Wilks' lambda, Hotelling's trace, and Roy's largest root)

Brazil) to 740 MBq (20 mCi) of DTPA (DTPATEC-S, SORIN BIOMEDICA S.p.A., Italy) in 5 m*l* of normal saline. This solution was placed in the nebulizer reservoir (Aerogama®, Medical, Porto Alegre, RS, Brazil) with an oxygen inflow of 9 liters/minute, which was inhaled by the patient for 3 minutes. Next, the patient was placed in the supine position over a gamma camera (Starcam 4000i, GE, EUA) and images were obtained every 20 seconds for a 30-minute period. Two regions of interest were defined: left lung and right lung, which were computer-drawn manually, and a time-activity curve was constructed. The negative inclination of each curve was defined as the clearance of each lung, by using the maximum and minimum values of the clearance. T½ was calculated in Excel for Windows software.

Statistics

Data were analyzed by SPSS for Windows (Statistical Package for Social Sciences) and are described as mean and standard deviation. ^{99m}Tc-DTPA clearance before chemotherapy, after each cycle, 3 weeks and 3 months after stopping chemotherapy, was compared one to the other by means of pairwise comparisons, and a variance analysis was applied with repeated measures at the 0.05 level. The three patients who received 270 mg of bleomycin did not undergo the same number of ^{99m}Tc-DTPA as the patients who received 360 mg of bleomycin. Whenever this took place, their T½ ^{99m}Tc-DTPA was obtained by calculating the mean values of the other patients at that moment.

RESULTS

Patient characteristics

Frequencies of age, gender, diagnosis and cumulative bleomycin dose are shown in Table 1. Most of the patients were males (10 patients) with testicular cancer (10 cases). There were 2 females (1 patient with ovary germ cell tumor and 1 patient with Hodgkin's disease). The mean age was 28.6 years (range 21–43). None of these patients were smokers or had pulmonary disease. They had showed no pulmonary symptoms 30 days before enrollment, had received no antineoplastic treatment prior to it, neither

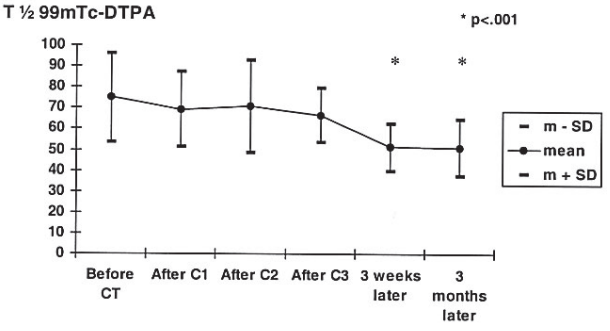

Fig. 1 Mean value of T½ ^{99m}Tc-DTPA scintigraphy.

thoracic irradiation nor thoracic surgery. Renal function was normal in all patients before and during treatment (Table 1).

Three patients received 270 mg of cumulative bleomycin dose, 1 patient received 215.2 mg of cumulative bleomycin dose and 8 patients received 360 mg of cumulative bleomycin dose.

Aerosol lung scintigraphy

All mean values of T½ ^{99m}Tc-DTPA scintigraphy are shown in Table 2. Two patients did not undergo ^{99m}Tc-DTPA scintigraphy in one of the scheduled measures because of compliance problems. Patient number 2 did not undergo the ^{99m}Tc-DTPA after C2 and patient number 5 did not undergo the ^{99m}Tc-DTPA at 3 months after chemotherapy. Every patient underwent approximately six ^{99m}Tc-DTPA scintigraphies. Three patients received 270 mg of total bleomycin dose and performed 5 ^{99m}Tc-DTPA scintigraphies. A total of 67 ^{99m}Tc-DTPA scintigraphies were performed during the study.

The mean value of T½ ^{99m}Tc-DTPA scintigraphy before treatment (74.93 (21.23)) was significantly higher than the mean value at the third month after treatment (51.00 (13.48)) ($p < 0.001$) as shown in Table 3 and Figure 1. There were no significant alterations in values of T½ ^{99m}Tc-DTPA before 171 mg of bleomycin. The first decrease observed occurred after the patient received 256.5 mg of bleomycin, which is equivalent to 3 cycles of BEP. This alteration was persistent and progressive until

the patient reached 347.9 mg of bleomycin. These alterations were still present three months after stopping treatment on multivariate tests on independent pairwise comparisons.

After treatment, only 1 patient (number 2) presented radiological signs of lung toxicity due to bleomycin. This patient underwent pulmonary biopsy but no pulmonary fibrosis was found. The patient received no corticoesteroid therapy and had spontaneous resolution. The other patients had no complications during chemotherapy, and completed the treatment with no problems. As of February 2002 no relapses have been described.

DISCUSSION

Bleomycin is an antitumoral antibiotic that may cause pulmonary epithelial injury even at low doses. There are many methods to evaluate the pulmonary epithelial membrane in animals. Nowadays the most used test is DLCO but it should be correlated with the clinical and radiological signs. The use of invasive tests might help in the diagnosis of pulmonary injury. Bronchoalveolar lavage and pulmonary biopsy can be performed when the diagnosis of pulmonary fibrosis remains questionable with clinical, radiographic, and pulmonary function tests. The mortality rate with an open lung biopsy is less than 1% but is not negligible.

Since 1977, technetium-99m diethylene triamine pentaacetic acid aerosol (^{99m}Tc-DTPA) scintigraphy has been used as a useful, sensitive and cheap method.[14] ^{99m}Tc-DTPA scintigraphy changes reflect a change in the permeability of the alveolocapillary barrier.[15] This test is a noninvasive method, which can be easily performed many times. ^{99m}Tc-DTPA scintigraphy can be a diagnostic tool for detecting early pulmonary injury in special clinical conditions, such as monitoring activity of pulmonary disease, and in the differential diagnosis between ARDS and cardiogenic pulmonary edema.[14,16–19]

The consensus panel of The National Heart, Lung and Blood Institute concluded that ^{99m}Tc-DTPA is a highly sensitive method for excluding pulmonary epithelial injury.[16]

The clearance of ^{99m}Tc-DTPA scintigraphy is altered in smokers but becomes normal 1 week after smoking cessation.[20–25] Terra-Filho et al., showed that there is an alteration in ^{99m}Tc-DTPA in patients using amiodarone, and that this method is useful as an early diagnostic tool.[26,27] The ^{99m}Tc-DTPA has 100% sensitivity and 80% specificity when compared to ^{67}Ga-citrate, and these altered values become normal 120 days after stopping the intake of amiodarone.[28] Furthermore, ^{99m}Tc-DTPA scintigraphy offers better results than Ga-67 scintigraphy.[29] ^{99m}Tc-DTPA scintigraphy can also be altered in patients who are receiving fluoxetine.[30]

Ugur et al., evaluated bleomycin pulmonary toxicity using ^{99m}Tc-DTPA. Twenty patients with germ cell tumor were treated with 4 cycles of etoposide, cisplatin and bleomycin. ^{99m}Tc-DTPA was performed before chemotherapy, after 180 mg and after 360 mg of bleomycin, and the values expressed in Kep (k% of decay min^{-1}) were significantly lower before chemotherapy, when compared to values of 180 mg and 360 mg of bleomycin. The authors concluded that ^{99m}Tc-DTPA can be a useful method to evaluate alveolocapillary injury during treatment with bleomycin, there being a cumulative effect.[31] Despite these results, Van Barneveld et al. described that ^{99m}Tc-DTPA did not show late pulmonary complications two years after treating 93 patients having disseminated testicular cancer with bleomycin containing chemotherapy.[32]

Our study differs from the study by Ugur because we performed ^{99m}Tc-DTPA scintigraphy before every cycle during chemotherapy up to the third month after stopping treatment. In our study, altered pulmonary epithelial permeability analyzed by ^{99m}Tc-DTPA scintigraphy can be demonstrated after 256.5 mg of bleomycin. After the third cycle of chemotherapy there is a fall in $T_{½}$ ^{99m}Tc-DTPA, and this fall is more pronounced after 347.9 mg of bleomycin and is present up to the third month after chemotherapy.

Based on ^{99m}Tc-DTPA scintigraphy it is correct to say that bleomycin has no effect on this permeability up to an average dose of 171 mg and an increase in pulmonary epithelial permeability is seen at a dose of 256.5 mg. However, whether this increase is related to clinical toxicity is uncertain. Further multicentric studies comparing ^{99m}Tc-DTPA to DLCO are necessary to assure the safest dose of bleomycin and the avoidance of a lung injury before using this method as a standard.

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