
SPECT Quantitation of Cobalt-57-Bleomycin to Predict Treatment Response and Outcome of Patients with Lung Cancer

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Our hypothesis is that the concentration of ^{57}Co -bleomycin (Co-bleo) in lung tumors reflects tumor cell kinetics and thus, prognosis. The relationship between the tumor concentration of Co-bleo measured in vivo by quantitative SPECT, response to chemotherapy and survival was investigated. **Methods:** Twenty patients with small-cell lung carcinoma (SCLC) and 49 patients with non-small-cell lung carcinoma (NSCLC) were studied. The concentration of Co-bleo was measured by SPECT in vivo in the tumor. The correlation between Co-bleo concentration in the tumor and the fraction of Co-bleo bound to DNA was investigated in an EMT6 murine tumor model and in samples of eight human tumors. **Results:** Tumors that did not respond to treatment showed a significantly higher Co-bleo concentration 8 hr after injection than tumors that responded ($5.83\% \pm 1.97\%$ ID/cc $\times 10^{-3}$ versus $2.55\% \pm 1.23\%$ ID/cc $\times 10^{-3}$, $p < 0.001$). Values of Co-bleo concentration of 2.97% ID/cc $\times 10^{-3}$ for SCLC and 2.72% ID/cc $\times 10^{-3}$ for NSCLC were found to best separate patients into short- and long-term survival groups. In the EMT6 murine tumor model, a good correlation was found between the concentration of Co-bleo in the tumor and the fraction of Co-bleo bound to DNA ($r = 0.75$). In human tumor samples, a good correlation was found between DNA-bound Co-bleo measured in vitro and the concentration measured in vivo by SPECT ($r = 0.85$). **Conclusions:** SPECT-measured Co-bleo concentration predicts the response to treatment and the outcome in patients with lung tumor by showing Co-bleo binding to DNA and tumor cell kinetics.

Key Words: cobalt-57-bleomycin; SPECT; DNA; lung cancer

J Nucl Med 1994; 35:1129–1133

Using a quantitative SPECT technique, a marked variability in the concentration of Co-bleo was noted among lung tumors of the same histology (1). It was suggested that the concentration of Co-bleo is a characteristic of each individual tumor. When following the patients, it became evident that a relationship exists between the tumor con-

centration of Co-bleo and patients' outcome. Also, in some tumors, the Co-bleo concentration measured over time showed a rapid decrease while in others, Co-bleo concentration remained high for a long time after injection. The latter tumors were associated with short-term patient survival.

Our hypothesis was that the concentration of Co-bleo late after injection reflects tumor cell kinetics and thus, prognosis. When the biodistribution of labeled Bleomycin was studied, it was shown that late after injection labeled bleomycin is mainly intracellular, located in the nuclear fraction of the tumor cells. It was also discovered that Co-bleo binds stably to DNA at sites similar to those of Fe-bleomycin, the biologically active form of bleomycin (2–4).

In the present study, the concentration of Co-bleo in the whole tumor, as measured in vivo by quantitative SPECT, was correlated with the fraction of Co-bleo bound to DNA in both the EMT6 murine tumor model and in samples of human tumors (1,5). To determine the relationship between Co-bleo concentration and prognosis, response to chemotherapy and survival were correlated with SPECT-measured Co-bleo uptake in 69 patients with lung cancer.

MATERIAL AND METHODS

SPECT Technique

We have used a previously described SPECT technique for in vivo quantitation of radiolabeled drug uptake in human tumors (5,6). The studies were performed using a digital gamma camera with a rotating gantry (Apex-415 ECT, Elscint, Haifa, Israel). A 64×64 -byte matrix was used and 2,000–4,000 counts were collected for each projection with a total of $1.5\text{--}2.4 \times 10^5$ counts for each study. Using a Hanning filter, the data were reconstructed by backprojection. A threshold of 0.43 (43% of the maximal counts) was used to measure the concentration in the tumor. This threshold was experimentally found to give the best correlation between actual and SPECT-measured concentrations both in phantom studies and in comparison of in vivo and in vitro measurements of human tumor specimens obtained during surgery (1,5). For each slice, the pixels containing counts that exceeded that of the threshold were used to calculate the concentration of the radiolabeled drug in the tumor.

Received Sept. 28, 1993; revision accepted Feb. 2, 1994.
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SPECT studies were performed 30, 120, 240 and 480 min after the intravenous injection of 2 mCi of Co-bleo. Commercial bleomycin (Blenoxan, Lundbeck, Copenhagen, Denmark) used for routine chemotherapy was labeled with ^{57}Co in our laboratory as previously described (7). The concentration in the tumor was expressed as the percent of injected dose per cubic centimeter (%ID/cc) of tumor tissue for each of the time points and was normalized for the patient's body surface area. The percent of change in concentration of Co-bleo in the tumor over time was calculated as following:

$$\frac{(\text{concentration at 30'} - \text{concentration at 480}')}{\text{concentration at 30'}} \times 100.$$

Tumor Response and Survival Studies

The concentration of Co-bleo was investigated before treatment in 20 patients with newly diagnosed SCLC. Sixteen patients with SCLC received a combination chemotherapy treatment consisting of cyclophosphamide, doxorubicin, vincristine (CAV) alternating with etoposide and cisplatin (VpP). The other four patients with SCLC were treated with doxorubicin, cyclophosphamide and etoposide (ACE). Forty-nine patients with NSCLC were investigated, including 19 patients with squamous-cell carcinoma, 22 patients with adenocarcinoma, 3 patients with large-cell carcinoma and 5 patients with undifferentiated carcinoma of the lung. Patients with NSCLC were not treated with chemotherapy.

The concentration of Co-bleo was investigated for a possible relationship with tumor response to chemotherapy in patients with SCLC. Response of the lung tumors to chemotherapy was determined by a follow-up CT examination. The tumors were classified into two groups: responders and nonresponders. Tumors that responded either disappeared or showed a reduction in tumor mass of at least 50% (complete response or partial response). Nonresponding tumors showed little or no change in tumor mass or tumor progression (8).

The relationship between Co-bleo concentration 8 hr after injection and patient survival was determined in both SCLC and NSCLC patients. Survival time was measured from the day of definite diagnosis to death. Co-bleo concentration values at 8 hr after injection, which best separated patients into short-term and long-term survival groups, were selected by an ROC curve analysis. Survival curves of low uptake (below the value) and high uptake (above the value) tumors were generated using the Kaplan-Meier method (9) and *p* values for survival comparison were obtained using the log rank statistics.

Correlation Between Whole-Tumor Co-Bleo and DNA-Bound Co-Bleo

The correlation between whole-tumor Co-bleo and DNA-bound Co-bleo was determined in an EMT6 murine tumor model. Tumor cells were implanted in the thigh muscles of 16 Balb C mice. The animals were killed 2–3 hr after the injection of Co-bleo. Cell suspensions of EMT6 tumor were obtained by disaggregation with DMSO buffer (5% dimethyl sulfoxide/250 mM sucrose/40 mM sodium-citrate, pH 7.6) at 4°C as described by Vindelov et al. (10). Isolation of nuclei and DNA was performed as described by Kono (11). Correction for DNA loss during preparation was achieved by measuring the DNA content in the tissue and the DNA content in the DNA-bleomycin fraction by the diphenylamine method.

The correlation between SPECT-measured Co-bleo concentra-

tion and *in vitro* measured DNA-bound Co-bleo was investigated in eight patients which were not included in the survival evaluation. These included five patients with a lung malignancy, two patients with lymphoma, and a patient with a lymph node metastasis of clear-cell carcinoma. SPECT studies were started 1 day prior to the surgery and completed on the day of surgery. The concentration of Co-bleo was measured at 30 min, 2 hr, 4 hr, 8 hr and approximately 24 hr after injection. A biexponential-fit curve was generated for the SPECT-measured concentration over time for each tumor. Tumor specimens obtained at surgery were placed on ice. The tissue was first disaggregated enzymatically by 0.2% trypsin solution at room temperature and then washed and centrifuged at 600G at 4°C. Isolation of nuclei and DNA and the determination of DNA-bound Co-bleo were performed as described for animal studies. The value of DNA-bound Co-bleo was then correlated with the value of SPECT concentration that was predicted by the fit curve at the same time the specimens were obtained.

RESULTS

Table 1 summarizes the clinical data and the tumor concentration of Co-bleo 8 hr after injection in 20 patients with SCLC. Fourteen patients responded to chemotherapy, for a response rate of 70%. Of the 14 tumors which responded to chemotherapy, 12 showed a rather rapid decrease in Co-bleo concentration over time. Nonresponding tumors showed either a slower decrease in the concentration over time or a concentration of Co-bleo that remained high or increased with time (Fig. 1). The percentage change in concentration over time in responders was $-41.96\% \pm 27.64\%$ as compared to $+1.53\% \pm 23.87\%$ in nonresponders ($p < 0.01$). The difference in uptake between nonresponders and responders was most significant at 480 min ($5.83\% \pm 1.97\% \text{ ID/cc} \times 10^{-3}$ versus $2.55\% \pm 1.23\% \text{ ID/cc} \times 10^{-3}$, $p < 0.001$). The difference was less significant at 30 min after injection ($5.60\% \pm 0.74\% \text{ ID/cc} \times 10^{-3}$ versus $4.19\% \pm 1.07\% \text{ ID/cc} \times 10^{-3}$, $p < 0.02$). In one patient, it was possible to measure Co-bleo concentration at the time of diagnosis when Co-bleo concentration was low and the tumor responded to chemotherapy. When the tumor relapsed, it showed a high late concentration and did not respond to chemotherapy (Fig. 2).

The relationship between Co-bleo concentration and survival was evaluated in 20 patients with SCLC and in 49 patients with NSCLC. The longest survival time in patients with SCLC was 27 mo and in patients with NSCLC was 42 mo. A ROC curve analysis was used to select the value of concentration at 8 hr after injection which best separated the patients into short-term and long-term survival groups. A value of $2.97\% \text{ ID/cc} \times 10^{-3}$ separated SCLC tumors into low-concentration and high-concentration tumors. There were eleven tumors with low concentrations and nine tumors with high concentrations of Co-bleo. The median survival time of patients with low-concentration tumors was 17 mo, while the median survival time of patients with high-concentration tumors was 9 mo. The survival curves of low- and high-concentration SCLC tumors are shown in

TABLE 1
Clinical Data and Co-bleo Concentration Eight Hours Postinjection in SCLC Patients

Patient	Age/sex	Stage	Co-bleo concentration	Chemotherapy	Response	Survival (mo)
1	60, F	ED	1.09	ACE	R	27
2	56, F	LD	1.15	CAV-VpP	R	21
3	55, M	LD	1.52	CAV-VpP	R	13
4	65, M	ED	1.71	CAV-VpP	R	14
5	85, M	ED	1.72	CAV-VpP	R	12
6	72, M	LD	2.24	CAV-VpP	R	17
7	66, F	ED	2.49	CAV-VpP	R	6
8	69, M	LD	2.59	CAV-VpP	R	26
9	60, M	LD	2.65	CAV-VpP	R	10
10	61, M	LD	2.87	CAV-VpP	R	24
11	76, M	LD	2.90	CAV-VpP	R	14
12	51, M	ED	3.08	CAV-VpP	NR	6
13	75, M	LD	3.10	CAV-VpP	R	10
14	55, M	LD	3.54	CAV-VpP	R	9
15	57, M	LD	3.98	CAV-VpP	NR	8
16	65, M	LD	5.09	CAV-VpP	NR	9
17	61, M	LD	6.14	ACE	R	16
18	66, M	LD	6.94	CAV-VpP	NR	12
19	46, M	ED	7.03	ACE	NR	2
20	72, M	ED	8.85	ACE	NR	5

ED = extensive disease; LD = limited disease; ACE = doxorubicin, cyclophosphamide, etoposide; CAV-VpP = cyclophosphamide, doxorubicin, vincristine alternative with etoposide and cisplatin; R = responder; and NR = nonresponder.

Figure 3. The curves differed significantly ($p < 0.01$) over a follow-up period of 12 mo.

For NSCLC patients, the value that separated short-term and long-term survival was $2.72\% \text{ ID/cc} \times 10^{-3}$. There were 31 tumors with low Co-bleo concentrations and 18 tumors with high Co-bleo concentrations. The median survival time of patients with low concentrations was 13 mo while the median survival time for patients with high concentrations was 8 mo. The survival curves of low- and high-concentration NSCLC tumors are shown in Figure 4. For a follow-up period of 12 mo the curves differed significantly ($p < 0.05$). After 12 mo from diagnosis there were 9 survivors with SCLC and 16 with NSCLC.

The correlation between the Co-bleo concentration in the whole tumor and DNA-bound Co-bleo in the EMT6

murine tumor model was $r = 0.75$ (Fig. 5). In eight human tumors, there was a good correlation ($r = 0.85$) between in vivo SPECT-measured concentrations of Co-bleo and in vitro measurements of DNA-bound Co-bleo performed at the same time (Fig. 6).

DISCUSSION

The correlation between tumor cell kinetics and prognosis has been investigated in various human tumors including lung carcinoma, lymphoma and breast and colon carcinoma (12-18). The labeling index (LI), which represents the proportion of tumor cells engaged in DNA synthesis

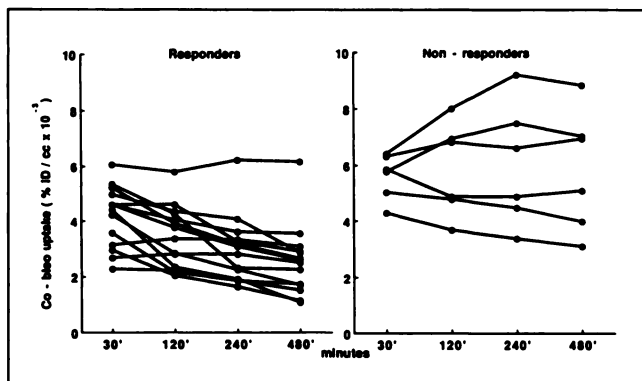


FIGURE 1. Tumor concentration over time curves of Co-bleo in SCLC patients responding and not responding to chemotherapy.

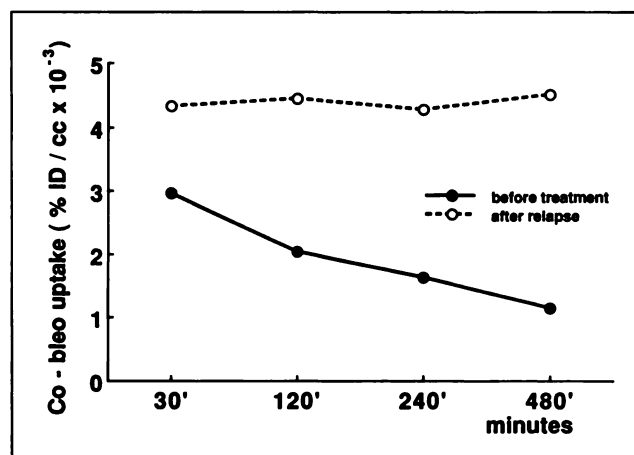


FIGURE 2. Co-bleo tumor concentrations in a patient with SCLC before treatment and after relapse of the disease.

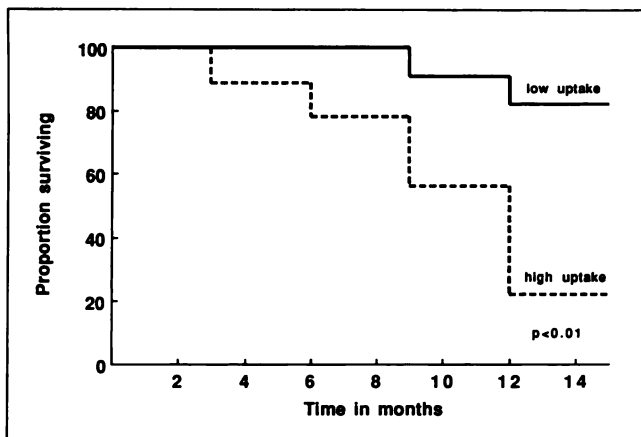


FIGURE 3. SCLC-survival curves of patients with low and high tumor uptake of Co-bleo.

(S phase fraction), and the DNA content are the parameters usually used to express tumor cell kinetics. These studies have indicated that tumors that show high LI and/or high DNA content are rapidly growing and are associated with poor prognosis and unresponsiveness to chemotherapy. Determination of the LI and of DNA content is obtained *in vitro* and requires extirpation of the tumor and multiple biopsy sampling. The LI can be obtained by autoradiography of specimens of tumor that have been exposed to H3-thymidine or iodo- or bromo-deoxyuridine (13-16). H3-thymidine is associated with a high radiation dose. *In vivo*, iododeoxyuridine shows a rapid dehalogenation in the liver. Both agents are not optimal for *in vivo* use.

In the present study, *in vivo* measured high concentrations of Co-bleo in tumors late after injection are shown to be associated with a poor prognosis. Survival time of patients with high tumor concentrations was significantly shorter than the survival time of patients with low concentrations. Patients with SCLC who did not respond to chemotherapy had significantly higher tumor concentrations of Co-bleo at 8 hr after injection in comparison to patients

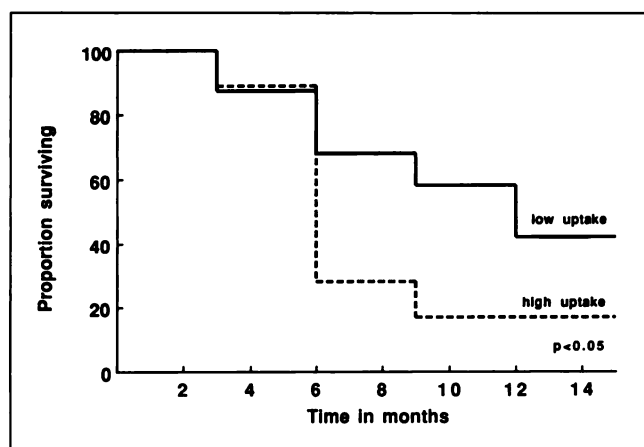


FIGURE 4. NSCLC-survival curves of patients with low and high tumor uptake of Co-bleo.

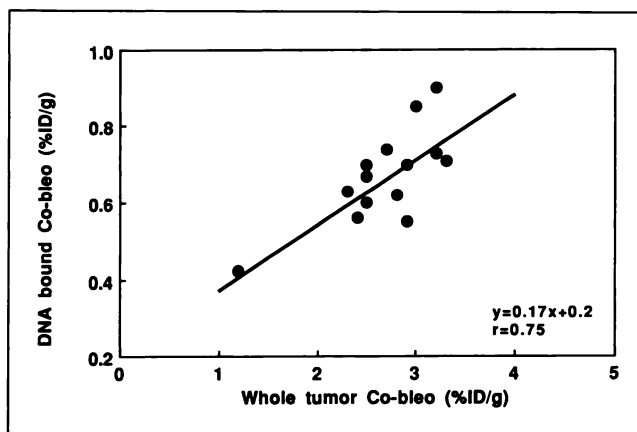


FIGURE 5. EMT6 correlation between whole-tumor Co-bleo and DNA-bound Co-bleo.

who responded to therapy. Differences in concentration between the response groups were less significant at 30 min after injection. These results suggest that early and late tumor concentration of Co-bleo reflect different characteristics of the tumor. Our hypothesis is that while the initial uptake of Co-bleo depends on properties of the tumor such as blood flow, tumor capillary permeability and extracellular volume, the concentration in the tumor late after injection, when the concentration in the blood and in the extracellular space is decreased, reflects DNA binding and tumor cell kinetics (2,19).

Labeled bleomycin has been shown in biodistribution studies to be intracellular, located mainly in the nuclear fraction of the tumor cells, four or more hours after injection; Co-bleo binds to the DNA molecule at similar sites as Fe-bleo, the cytotoxically active form of bleomycin. Chelation of bleomycin with cobalt has been shown to enhance the binding of bleomycin-DNA and to increase the binding stability (2-4). In this study, a good correlation was found between the Co-bleo concentration in the whole tumor, as measured by SPECT, and the fraction of Co-bleo bound to

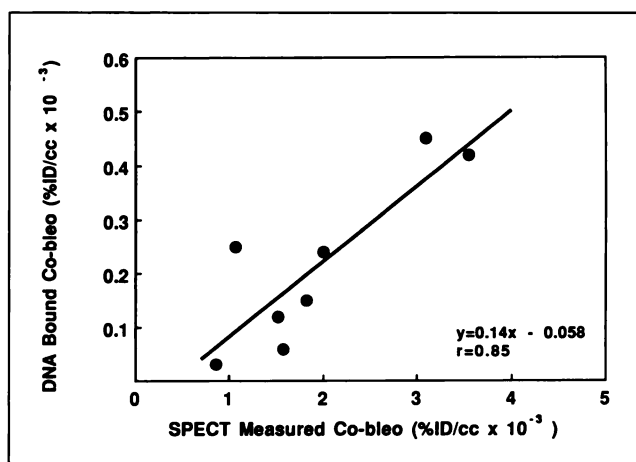


FIGURE 6. Human tumors correlation between SPECT-measured and DNA-bound Co-bleo.

DNA. This data indicate that Co-bleo concentration may be considered as an in vivo indicator of cell kinetics. The long half-life of ^{57}Co should be considered and strict radiation safety protocol should be practiced in the clinical use of Co-bleo.

In summary, Co-bleo uptake is of predictive value in determining tumor response to chemotherapy and the outcome of patients with lung cancer. Tumors that show high concentrations of Co-bleo late after injection respond poorly to chemotherapy and are associated with shorter survival periods than tumors showing lower in vivo Co-bleo concentrations.

ACKNOWLEDGMENTS

The authors wish to thank Dr. S. Parmett for reviewing the manuscript. This study was supported by grants from the Israeli Cancer Association; the chief scientist of the Israeli Ministry of Health; and the Technion and Rappaport Institute, Haifa, Israel.

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