Anti-Cancer Drugs, 2, pp. 555–563

Pharmacokinetic behavior of [^{57}Co]Bleomycin liposomes in mice: comparison with the unencapsulated substance

Iduna Fichtner,[CA] Dieter Arndt, Regina Reszka and J. Gens

I Fichtner, D Arndt and R Reszka are at the Central Institute of Cancer Research, Lindenberger Weg 80, 0-1115 Berlin-Buch, Germany. Fax: (372) 3495511. J Gens is at the Central Institute of Nuclear Research, Rossendorf, Germany.

The distribution and excretion of [^{57}Co]Bleomycin, dissolved in saline or encapsulated in liposomes, was studied in normal or tumor-bearing [P388 leukemia, reticulum cell sarcoma (RS)] mice. The free substance is cleared relatively quickly from the organism both after intravenous and intraperitoneal administration ($t_{1/2}$ 0.17 and 2.41 h, respectively), and is excreted predominantly via the urinary tract. In contrast, following entrapment in small unilamellar vesicles (SUV) or multilamellar vesicles (MLV), the ^{57}Co radioactivity remains 7- to 30-fold longer in the blood stream and is detectable in considerable amounts in liver, spleen, lung and tumor of the RS model even after 48 h. Concomitantly, the renal excretion is diminished to about 50% of the free drug and the feces excretion is slightly increased, possibly due to the higher concentrations in the liver. Whereas the renal levels of radioactivity were similar with all application forms of [^{57}Co]Bleomycin, there were marked differences in all the other tissues studied. After administration of SUV there was a higher activity in liver, brain and tumor, whereas MLV were more concentrated in spleen and lung. Therapeutic experiments confirmed the favorable results obtained with liposomes. While the free Bleomycin in the P388 leukemia had only a moderate influence on the lifetime of the animals with a treated/control value of 111%, encapsulation of the drug in SUV or MLV improved the results to 194 and 167%, respectively. In the intramuscular transplanted RS model, the SUV in a day 1 schedule had the same effect on tumor growth as the free drug in a day 1–4 schedule. These favorable results obtained with Bleomycin-containing liposomes make this drug formulation attractive for clinical use.

Key words: Bleomycin, multilamellar vesicles, pharmacokinetic behavior, small unilamellar vesicles.

[CA] Corresponding Author

Introduction

Bleomycin is an established glycopeptide antibiotic of proven antineoplastic activity in human tumors, e.g. head and neck cancers, testes and cervix carcinomas and lymphomas.[1–4] The most prominent side effect of this drug is the production of lung fibrosis in about 10% of patients. Its pharmacokinetic behavior is characterized by hydrolytic inactivation and a relatively fast elimination via the urinary tract. Various experimental and clinical studies have examined how to prolong the bioavailability of Bleomycin in the organism and, hence, to improve the therapeutic effectiveness. A number of research groups[5–7] stated that by a continuous infusion of Bleomycin in murine tumor models, the antineoplastic activity was elevated and the lung toxicity diminished. Intratumoral or intramuscular injection of Bleomycin–oil suspensions[7–9] led to a higher antitumor effect in experimental systems, prolonged serum levels and delayed renal excretion. This application form also showed favorable results in some clinical studies[9–13] but is not available for general use. Another possibility for the generation of a depot form of a normally very fast eliminated drug is encapsulation in liposomes.[14] Sur and Roy,[15] who were the first to use Bleomycin-containing liposomes in an experimental tumor model (Ehrlich ascites carcinoma), noticed higher increase in life span values and more cured animals in comparison with the free drug. The best effects were obtained with positively charged liposomes. Gabizon *et al.*[16] used [^{67}Ga]deferoxamine and ^{111}In-labeled Bleomycin in two

murine and one human tumor model, and registered a 20- to 40-fold tumor accumulation when using special liposomal preparations. Firth *et al.* noticed a sustained release effect following intracerebral injection of Bleomycin in reverse phase evaporation vesicles in rats[17] or patients with gliomas.[18] We have registered a remarkable improvement of therapeutic results in P388 murine leukemia when Bleomycin in liposomal form was administered to the animals. We wondered if this effect could be due to different pharmacokinetic behavior of the drug in liposomes compared with the free form. We therefore decided to use ^{57}Co-labeled Bleomycin to examine this question. We administered the substance, free or liposomally encapsulated, both intraperitoneally and intravenously and studied the disposition in blood and different organs in normal or tumor-bearing [P388, reticulum cell sarcoma (RS)] mice.

Materials and methods

Animals and tumors

P388 leukemia and a newly discovered RS line[19] were used as tumor models. The leukemia was transplanted intraperitoneally (5×10^5 ascitic cells) in female C57B1/6 × DBA/2 (BDF_1) mice. RS was inoculated intramuscularly in female Bln:NMRI mice. All animals had a body weight of 20 ± 0.5 g at the beginning of the experiments. They were held under conventional conditions with food and water *ad libitum*. In the experiments in which urine and feces were collected, the animals lived in metabolic cages, otherwise they were kept in normal plastic cages. Three to four mice were used for the determination of each value.

Substances

Bleomycin was purchased from Fa. Mack (Germany), bleomycin A_2 55–70%, bleomycin B_2 25–32%, and was always freshly dissolved in physiologic saline. ^{57}Co-labeling was performed according to Grove *et al.*,[20] chromatographic examination (according to Grove) at start 0%, [^{57}Co]bleomycin A 53.8%, [^{57}Co]bleomycin B 46.2%, $^{57}Co^{2+}$ 0%. Unlabeled or [^{57}Co]Bleomycin was encapsulated in small unilamellar vesicles (SUV) or multilamellar vesicles (MLV). For this purpose a lipid mixture (egg-phosphatidylcholine : cholesterol : dicetyl-phosphate; 1:1:0.1 molar ratio), starting from 1 mmol phosphatidylcholine, was suspended in 14 ml phosphate buffered saline (PBS) pH 7.2. PBS contained the drug (45 mg cold Bleomycin and 19 mg hot [^{57}Co]Bleomycin at 1.85 MBq). The mixture was shaken overnight. For SUV production the MLV suspension was sonicated for a total of 16 min in an ice bath, using a probe system (Branson B 15 sonifier) with a 50% pulsed input. Unencapsulated substance was removed from the MLV or SUV by dialysis (24 h, four changes of buffer). The diameter of the SUV was 50–70 nm; the size of the MLV was 15% smaller than 1200 nm, 65% between 1200 and 3000 nm, and 20% larger than 3000 nm; the Bleomycin content was 1.3–2.0 mg/ml; lipid concentration (PC) was 0.076 mol/l; and the radiochemical concentration was 0.03–0.08 MBq/ml.

Experiments

Following intraperitoneal or intravenous administration of 40 mg/kg Bleomycin, corresponding to an activity of about 0.037 MBq (10 μCi), blood was collected 5 min, 10 min, 30 min, 1 h, 4 h, 24 h and 48 h later, respectively, from the retro-orbital venus plexus, and the corresponding radioactivity/ml was determined. The application volume was 0.4–0.8 ml. A dilution effect of blood due to this relatively high volume must be considered. Groups (three mice each) of treated animals were killed by cervical dislocation after 1, 4, 24 and 48 h; liver, spleen, lung, kidney and brain were removed; and the radioactivity was measured in whole organs. In the RS model, where the substances were injected intraperitoneally on day 21, the activity in the tumor was also determined. For the evaluation of antineoplastic activity, P388 tumor-bearing mice were treated intraperitoneally on day 1 with the corresponding substance, the median survival time (MDST) of the animals was registered and calculated in percent relation to the saline treated control group [treated/control (T/C)%]. Tumor growth of intramuscularly transplanted tumor was measured with a caliper-like mechanical instrument and tumor volume was then calculated.

Intravenous infusion of Bleomycin (40 mg/kg/day) was managed by means of an infusion pump (463A, Medipan, Warszaw). The treatment volume for free Bleomycin was 0.2 ml/20 g body weight, the liposome volume varied between 0.4 and 0.8 ml/20 g body weight depending on the Bleomycin content of the different preparations.

Radioactive measurements

^{57}Co radioactivity was measured in a scintillation counter (γ-Set 500, ICN, Germany) over 5 min without further preparation of blood and tissues.

Calculations

The pharmacokinetic data were calculated by means of a computer program (FORTRAN, ESER computer).[21] Significant differences between values were proved by the Mann–Whitney *U*-test.

Results

Table 1 shows one of several experiments concerning the antineoplastic activity of different Bleomycin preparations in the P388 leukemia. The substance in its free form had only a slight effect on the lifetime of the animals (group A). After a 48 h infusion this minimal activity could not be elevated (group G). Encapsulation in liposomes decisively improved the T/C values. There was no significant difference between MLV and SUV (group B and C). [^{57}Co]Bleomycin in SUV (group F) had a comparably good activity, while the unlabeled Bleomycin, both in free (group D) and in liposomal form (group E), did not prolong the lifetime of the tumor-bearing animals. This apparent discrepancy can be explained by the fact that for the preparation of [^{57}Co]Bleomycin SUV a 1:3 dilution with unlabeled Bleomycin was used. We suppose that this amount of unlabeled drug was enough to cause the relatively good effectiveness.

Table 1. Therapeutic effectiveness of different Bleomycin preparations in the P388 leukemia[a]

Group	Substance	T/C (%)	Cures/ total	Body weight change (%)
A	Bleomycin	111[b]	0/10	1
B	Bleomycin in SUV	194[b,c]	2/10	−3
C	Bleomycin in MLV	167[b,c]	1/10	−3
D	Co-Bleomycin	100	0/10	5
E	Co-Bleomycin in SUV	100	0/10	7
F	[^{57}Co]Bleomycin in SUV	183[b,c]	0/10	—
G	Bleomycin as 48 h infusion	118[b]	0/ 9	−2

[a] Tumor cells (0.5 Mio) were transplanted i.p. on day 0; 24 h later intraperitoneal treatment followed.
[b] Significant versus controls ($p = 0.05$).
[c] Significant versus free Bleomycin ($p = 0.05$).

The blood levels of ^{57}Co after intravenous application of ^{57}Co-labeled Bleomycin in free form, in SUV or MLV can be seen in Figure 1. The free Bleomycin is eliminated from the blood stream relatively quickly, so that after only 1 h minimal amounts could be detected. In contrast, Bleomycin in liposomes resulted in 6- to 10-fold higher peak levels and a delayed clearance. Surprisingly, similar half-lifes were determined with SUV and MLV in this experiment regardless of the different sizes of the vesicles. Pharmacokinetic parameters of intravenous and intraperitoneal application routes of different experiments can be seen in Table 2. Considering the elimination half-times these values are lower after intravenous than after intraperitoneal administration of free drug. This means that by using an extravasal route a certain depot effect can be reached. However, liposomal encapsulation resulted in a 7- to 30-fold prolongation of the elimination half-times. MLV were cleared in experiment 1 at approximately the same speed as SUV, in experiments 2 and 5 at a higher speed. Comparing the AUC values after intravenous or intraperitoneal administration of SUV, respectively, about 35% or 70% of the liposome dose was absorbed from the peritoneum of tumor-free or tumor-bearing mice.

In the BDF_1 mice the AUC values were similar for SUV and MLV, whereas in the RS-bearing mice SUV had an about 5-fold higher AUC and an about 2.5-fold higher distribution volume. In general, comparisons between different experiments should be done with caution because of the relatively small number of animals per value. Animals were killed

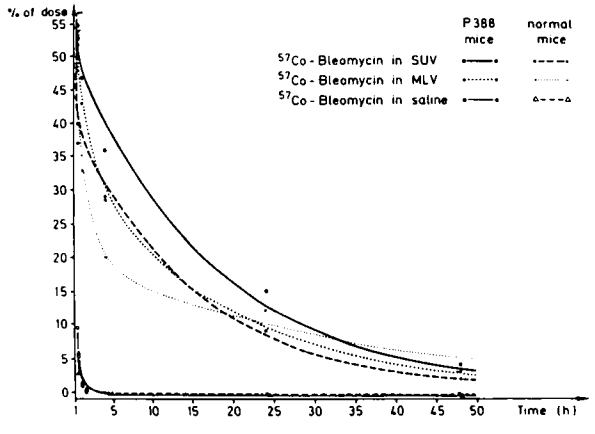

Figure 1. Computer fitted curves of blood levels of radioactivity between 5 min and 48 h after i.v. application of [^{57}Co]Bleomycin in SUV, MLV and saline. Each point represents the mean of four values. P388 mice received 0.5 Mio tumor cells i.p. 24 h before treatment.

Table 2. Pharmacokinetic parameters of intravenously or intraperitoneally administered Bleomycin in saline, SUV or MLV

Experiment no.	Mice	Tumor	Substance	Administration	AUC (μg h/ml)	Volume of distribution (ml)	Clearance (ml/min)	$t_{1/2}$ (h)
1	BDF_1	—	Bleomycin in SUV	i.v.	4904.8	962	41.2	6.50
			Bleomycin in MLV		5926.4	1700	19.6	8.48
			Bleomycin in saline		19.2	7325	5425.2	0.17
2	BDF_1	P388	Bleomycin in SUV	i.v.	7092.8	785	45.1	12.06
			Bleomycin in MLV		5496.8	1019	36.0	7.93
			Bleomycin in saline		104.0	21518	327.8	0.63
3	BDF_1	—	Bleomycin in SUV	i.p.	1747.2	3458	168.0	14.29
			Bleomycin in saline		83.2	13318	3829.0	2.41
4	BDF_1	P388	Bleomycin in SUV	i.p.	5002.4	1429	52.6	18.80
			Bleomycin in saline		35.2	15750	9013.0	1.21
5	NMRI	RS	Bleomycin in SUV	i.p.	4940.8	2225	61.7	24.99
			Bleomycin in MLV		1060.0	890	60.4	9.76
			Bleomycin in saline		19.2	20917	16728.0	0.87

48 h after intravenous application and the remaining activity was measured in various organs (Table 3). Consistent with the fast clearance from the blood stream, free Bleomycin could be found after this relatively long period only in liver and kidney in amounts worth mentioning. Similar levels were also noticed in the kidney after treatment with drug-containing SUV or MLV, while in all other organs higher concentrations were registered following liposomes. Especially impressing are the about 100-fold increased values in the liver, when the drug was given in liposomes instead of free form. In the lungs MLV are 14- to 32-fold more concentrated than SUV after 48 h and both vesicles are considerably higher than the free drug. Surprisingly, a remarkable elevation of radioactivity content in the brain resulted from the treatment with liposomes, from MLV more than from SUV. Results of two experiments in normal and P388-bearing mice with intraperitoneal application of Bleomycin in saline or SUV are summarized in Figures 2–5. The results from the intravenous route confirmed the differences in blood levels and tissue concentrations of free and liposomal drug. Again, similar amounts were found during the whole test period in the kidneys, while in all other organs and the blood stream [^{57}Co]Bleomycin SUV were retained longer than the free substance. Highest blood concentrations in tumor-bearing animals were measured 4 h after treatment with liposomes and 30 min after giving the saline solution with peak levels of 18.8 and 5.1% of administered dose, respectively.

The higher activity levels in liver, spleen, lung

Table 3. Organ radioactivity 48 h after intravenous treatment with [^{57}Co]Bleomycin in saline, SUV or MLV (each value is the mean ± SD of four animals)

	Percentage of dose ± SD					
	[^{57}Co]Bleomycin in SUV		[^{57}Co]Bleomycin in MLV		[^{57}Co]Bleomycin in saline	
	P388	normal mice	P388 mice	normal mice	P388 mice	normal mice
Liver	16.2 ± 0.7[a,b]	16.9 ± 2.1[a,b]	8.3 ± 2.5[a]	12.6 ± 0.06	0.62 ± 0.06	0.38 ± 0.09
Spleen	4.0 ± 0.3[a]	2.3 ± 0.8[a,b]	5.2 ± 1.2[a]	6.7 ± 1.3[a]	0.04 ± 0.008	0.01 ± 0.005
Lung	0.5 ± 0.1[a,b]	0.25 ± 0.03[a,b]	7.2 ± 1.2[a]	8.1 ± 0.5[a]	0.02 ± 0.007	0.008 ± 0.001
Kidney	0.38 ± 0.04[a,b]	0.39 ± 0.04[a]	0.26 ± 0.03	0.4 ± 0.2[a]	0.24 ± 0.08	0.15 ± 0.02
Brain	0.06 ± 0.01[a,b]	0.04 ± 0.009[a,b]	0.21 ± 0.06[a]	0.3 ± 0.07[a]	0.002 ± 0.0	0.001 ± 0.001

[a] Significant versus free [^{57}Co]Bleomycin ($p = 0.05$).
[b] Significant between SUV and MLV ($p = 0.05$)

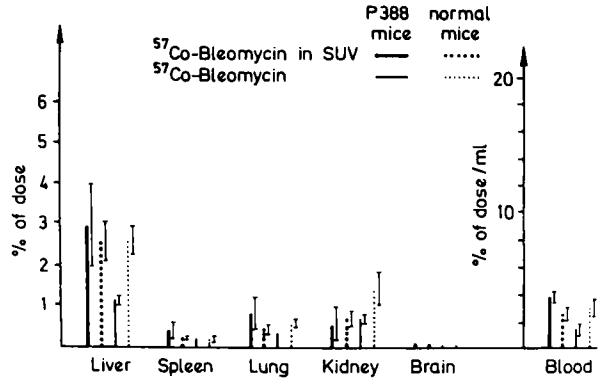

Figure 2. Radioactivity in different organs measured 1 h after intraperitoneal treatment of P388-bearing or normal mice with [^{57}Co]Bleomycin in SUV or saline. Each value is the mean of three mice.

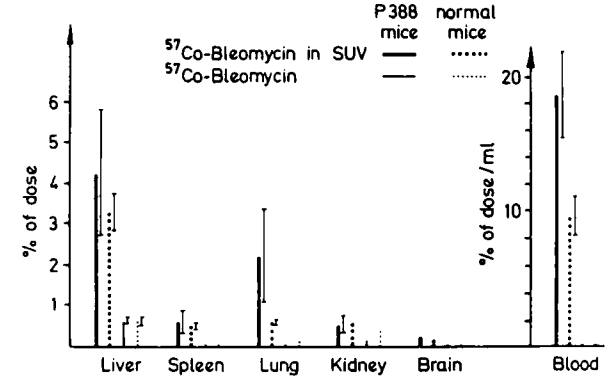

Figure 3. Radioactivity in different organs measured 4 h after intraperitoneal treatment of P388-bearing or normal mice with [^{57}Co]Bleomycin in SUV or saline. Each value is the mean of three mice.

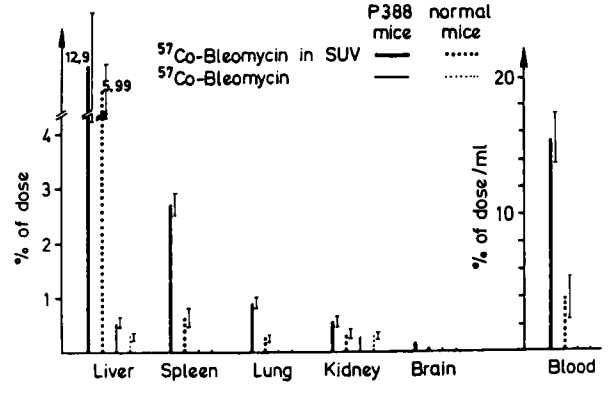

Figure 4. Radioactivity in different organs measured 24 h after intraperitoneal treatment of P388-bearing or normal mice with [^{57}Co]Bleomycin in SUV or saline. Each value is the mean of three mice.

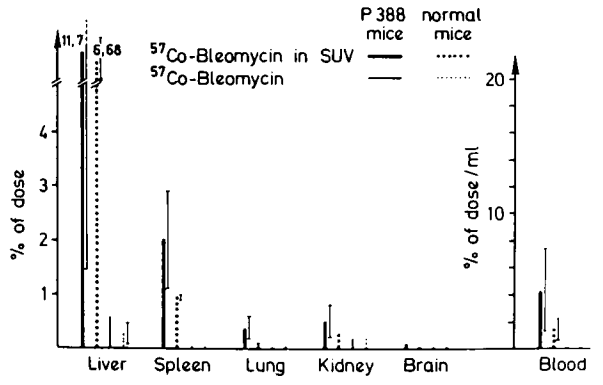

Figure 5. Radioactivity in different organs measured 48 h after interperitoneal treatment of P388-bearing or normal mice with [^{57}Co]Bleomycin in SUV or saline. Each value is the mean of three mice.

and brain of tumor-bearing mice compared with normal mice corresponded to the higher concentrations in the blood stream. The question whether these differences are really due to the presence of leukemic cells in the organism or are deviations between the two experiments cannot be answered.

Around 20.6% of dose after [^{57}Co]Bleomycin SUV and 45.3% of dose following free drug treatment of tumor-bearing animals were excreted via the urinary tract. In contrast, the feces excretion increased from 0.55% of the dose (Bleomycin in saline) to 6.63% of the dose (Bleomycin in SUV) within 24 h.

We used a solid reticulum cell sarcoma (RS) as a second tumor model. Therapeutic effectivity of Bleomycin in SUV compared to Bleomycin in saline can be seen in Table 4. Unfortunately, no treatment modality in this experiment prolonged the lifetime of animals in a significant way. However, when administering the drug once on day 1 in SUV, the tumor volume inhibition was better than after the free drug treatment in the same schedule and corresponded to a therapy over 4 days. This may be due, at least in part, to the higher blood levels (Figure 6) and tumor concentrations (Figures 7–10) measured with [^{57}Co]Bleomycin liposomes. Two- or 4-fold higher peak concentrations in blood were registered, when using the drug in MLV or SUV, respectively. The appearance of maximal levels was delayed from 10 min (saline) to 4 h (SUV) or 24 h (MLV), respectively. From the parallel decline during the last measured phase of blood levels we conclude that the still available MLV and SUV had a similar stability and were cleared with the same speed. During the whole time, higher tissue concentrations following liposome treatment could

Table 4. Antineoplastic activity of bleomycin in the RS model

Group	Number of animals	Treatment	Schedule (days)	Dose (mg/kg)	MDST (days)	T/C (%)	Tumor volume on day 18 (% of controls)
A	10	Bleomycin	1	20	33.5	85	82
B	10	Bleomycin in SUV	1	20	40.0	101	50[a]
C	10	Bleomycin	1–4	20	42.5	107	27[a]
D	10	Bleomycin in SUV	1–4	20	43.0	109	35[a]
E	10	physiological saline	1–4		39.5		

[a] Significant versus controls ($p = 0.05$).

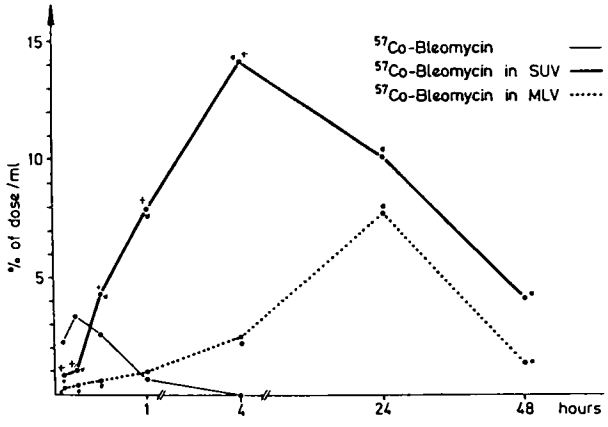

Figure 6. Blood levels of ^{57}Co after treatment of RS tumor-bearing mice with [^{57}Co]Bleomycin in SUV, MLV or saline. Each value is the mean of three mice. (*) Significant versus free drug. (+) Significant between MLV and SUV ($p = 0.05$).

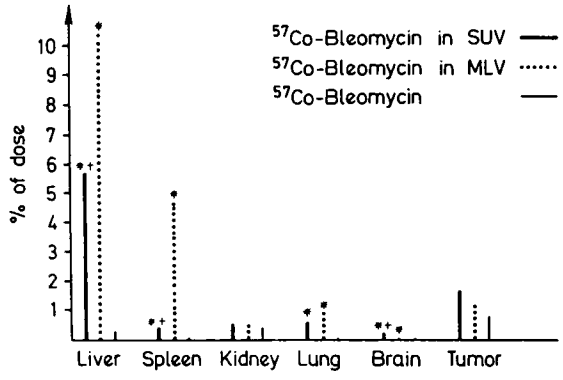

Figure 8. Radioactivity in different organs and in tumors measured 4 h after intraperitoneal treatment of RS-bearing mice with [^{57}Co]Bleomycin in SUV, MLV or saline. Each value is the mean of three mice. (*) Significant versus free drug. (+) Significant versus MLV and SUV ($p = 0.05$).

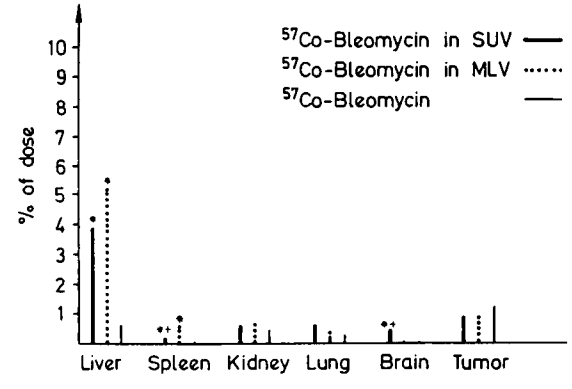

Figure 7. Radioactivity in different organs and in tumors measured 1 h after intraperitoneal treatment of RS-bearing mice with [^{57}Co]Bleomycin in SUV, MLV or saline. Each value is the mean of three mice. (*) Significant versus free drug. (+) Significant versus MLV and SUV ($p = 0.05$).

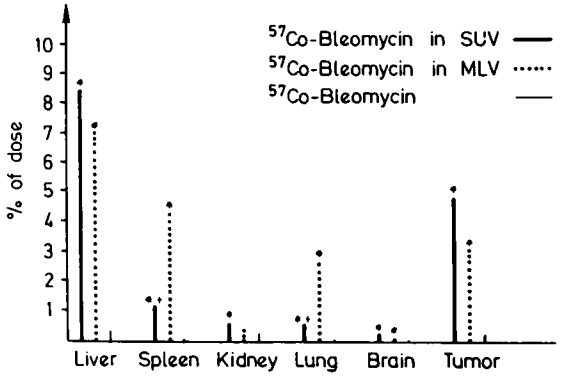

Figure 9. Radioactivity in different organs and in tumors measured 24 h after intraperitoneal treatment of RS-bearing mice with [^{57}Co]Bleomycin in SUV, MLV or saline. Each value is the mean of three mice. (*) Significant versus free drug. (+) Significant versus MLV and SUV ($p = 0.05$).

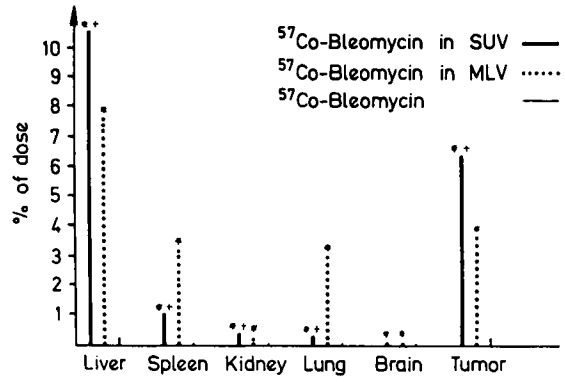

Figure 10. Radioactivity in different organs and in tumors measured 48 h after intraperitoneal treatment of RS-bearing mice with [^{57}Co]Bleomycin in SUV, MLV or saline. Each value is the mean of three mice. (*) Significant versus free drug. (+) Significant versus MLV and SUV ($p = 0.05$).

be found in liver, spleen, lung, brain and tumor, while in the kidney similar levels were registered as with free Bleomycin. Interestingly, we observed a change in distribution between MLV and SUV. At earlier times (1 and 4 h) higher liver concentrations were measured after MLV treatment, later (24 h and 48 h) higher liver concentrations were found after SUV treatment. We suppose that the liver uptake at earlier times is due to a capture of liposomes by the RHS, which occurs faster or at a higher degree with MLV. The values measured at later periods are possibly the result of the accumulation in the liver parenchyma, which is more pronounced with SUV[22]. In spleen and lung, higher activity levels were registered during the whole test period following MLV treatment. On the contrary, in brain and tumor tissues there was a preferred enrichment of SUV. Because of the higher tumor affinity we decided to use Bleomycin in SUV for the determination of therapeutic activity (Table 4). The 24 h urinary excretion for [^{57}Co]Bleomycin in SUV, MLV or saline was 0.9, 13.5 and 49.1% of the dose administered; the corresponding feces excretion was 19.3, 8.7 and 3.2%, respectively.

Discussion

Bleomycin forms a chelate complex with ^{57}Co which is relatively stable under physiologic conditions[23,24] and in the urine.[24] This labeled complex has a higher affinity for tumor tissue than other marker Bleomycins or the unlabeled drug[20,25–27] and was, therefore, proposed for diagnostic purposes,[28–30] especially for the scintigraphic imaging of liver metastases. We used this complex as a model to examine the pharmacokinetic behavior of a liposome encapsulated drug. In all experiments we noticed a marked prolongation of elimination half-time, higher peak levels, and a longer retention in such tissues as liver, spleen, lung, brain, tumor and in the blood, when Bleomycin was encapsulated in SUV or MLV. Concomitantly, the relatively fast urinary elimination of the free substance is slowed down. However, a slight increase in feces excretion, possibly caused by the higher liver enrichment, could be found. We conclude that the amounts of radioactivity measured after administration of the liposome-encapsulated drug are largely, if not entirely, due to the presence of intact vesicles in these organs or in the blood. Otherwise the breakage of liposomes and the leakage of [^{57}Co]Bleomycin would have led to a fast clearance of the marker from the organism as registered for the free substance. In general MLV had a more pronounced affinity for spleen and lung, whereas SUV had a high affinity for liver and tumor tissues. For that reason we used SUV in therapeutic experiments in two tumor models. The P388 leukemia is a model tumor in which tumor cells settle within 24 h after inoculation in liver, spleen, lung and brain of the animals. As in this case, the pharmacokinetic studies allowed no clear differentiation between normal and tumor tissues, we used normal, tumor-free mice for comparison. Free [^{57}Co]Bleomycin revealed no definite difference between these two kinds of mice, so that a special affinity of the marker to tumor cells could not be concluded from our experiments. However, higher blood levels after intravenous and intraperitoneal application, and increased liver, spleen, lung and brain concentrations after intraperitoneal application of SUV in tumor-bearing animals compared with normal mice led us to suppose a certain affinity for these tumor-cell-containing tissues. This is confirmed by the results of the therapeutic experiment. The slight effectivity of Bleomycin in this model, which is also known from the literature,[31,32] can be decisively improved when giving the drug in liposomes. Even a 48 h infusion of the saline soluted substance did not result in a comparable good effect.

The ineffectiveness of the unlabeled Co–Bleomycin, both in free and in liposomal form, can be explained by the fact that the relatively strong linkage between cobalt and the antibiotic[33] prevents the formation of the Fe(II) complex normally taking place under physiologic conditions. This complex

is responsible for the DNA strand scissions and the cytotoxic action.[23,34–36] The lack of antineoplastic activity is independent of the binding capacity of the Bleomycin molecule to DNA, which is caused by the intercalation of the planar bithiazole moiety and electrostatic attraction of the terminal amine.[37] Therefore, we conclude that in spite of the absence of cytotoxicity of the cobalt complex, the distribution pattern in the organism can be transferred to the situation of the cobalt-free Bleomycin normally used in cancer chemotherapy. Unambiguous evidence for the active drug in the different tissues and cells of the organism can be obtained only by using the unlabeled substance with other detection method, e.g. high performance liquid chromatography.

The favorable distribution pattern of liposomes, especially SUV, in the second, solidly growing tumor model supports our opinion that Bleomycin–liposomes could be a valuable drug carrier complex in cancer patients.

Acknowledgments

We thank Dr H Schmidt for performing the radioactive measurements, Dr Bache for the computer processing of pharmacokinetic data and Mrs M Becker for her excellent technical assistance in animal experiments.

References

1. Carter SK, Crooke ST, Umezawa H, eds. *Mechanism of Bleomycin: Current Status and New Developments.* New York: Academic Press 1978.
2. Carter SK, Bleomycin: More than a decade later. In: Sikic, RI, Rozenczweig M, Carter SK, eds. *Bleomycin Chemotherapie.* New York: Academic Press; **3**, 1985.
3. Crooke ST. Bleomycin—an overview. *Cancer Chemother* 1981; **III**: 97.
4. Evans WE, Yee GC, Crom WR. Clinical pharmacology of bleomycin and cisplatin. *Drug Intell Clin Pharm* 1982; **16**: 448–58.
5. Sikic BI, Collins JM, Mimnaugh EG, *et al.* Improved therapeutic index of bleomycin when administered by continuous infusion in mice. *Cancer Treat Rep* 1978; **62**: 2011–7.
6. Peng Y-M, Alberts DS, Cheng H, *et al.* Antitumor activity and plasma kinetics of bleomycin by continuous and intermittent administration. *Br J Cancer* 1980 *41*: 644–51.
7. Urano M, Koike S, Ando K, *et al.* Effect of continuous bleomycin treatment and of oil-suspended bleomycin on experimental tumor growth. *Jpn J Antibiot* 1976; **29**: 720–4.
8. Abe F, Tsubosaki M, Tanaka T, *et al.* Studies of absorption, excretion, and organ distribution of bleomycin oil suspension. *Jpn J Antibiot* 1976; **29**: 826–32.
9. Bier J, Handrock M, Matthias R. Intratumorale Chemotherapie mit Bleomycin-Emulsion: Tierexperimentelle Untersuchungen und klinische Anwendung. *Laryng Rhinol Otol* 1982; **61**: 577–81.
10. Davy M, Paus E, Lehne G. A pharmacokinetic evaluation of IM administration of bleomycin oil suspension. *Cancer Chemother Pharmacol* 1985; **14**: 274–6.
11. Slevin ML, Harvey VJ, Aherre GW, *et al.* Delayed-release bleomycin. Comparative pharmacology of bleomycin oil suspension and bleomycin in saline. *Cancer Chemother Pharmacol* 1984; **13**: 19–25.
12. Sugimura M, Horibata K, Shiba R, *et al.* Experimental and clinical studies of local application of solid and oil Bleomycin for the treatment of oral cancer. *J Maxillofacial Surg* 1981; **9**: 26–34.
13. Takahashi T, Heda S, Kono K, *et al.* Attempt of local administration of anticancer agents in the form of fat emulsion. *Cancer* 1976; **38**: 1507–11.
14. Juliano RL, Stamp D. Pharmacokinetics of liposome-encapsulated anti-tumor drugs. Studies with vinblastine, actinomycin D, cytosine arabinoside and daunomycin. *Biochem Pharmacol* 1978; **27**: 21–9.
15. Sur R, Hasra B, Roy K, *et al.* Enhancement of the activity of bleomycin on Ehrlich ascites carcinoma cells by liposomal encapsulation. *Indian J Exp Biol* 1984; **22**: 115–9.
16. Gabizon A, Price DC, Huberty J, *et al.* Effect of liposome composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies. *Cancer Res* 1990; **50**: 6371–82.
17. Firth G, Oliver AS, McKeran RO. Studies on the intracerebral injection of bleomycin free and entrapped within liposomes in the rat. *J Neurol Neurosurg Psychiat* 1984; **47**: 585–9.
18. Firth G, Firth M, McKeran MO, *et al.* Application of radioimmunoassay to monitor treatment of human cerebral gliomas with bleomycin entrapped within liposomes. *J Clin Pathol* 1988; **41**: 38–43.
19. Fichtner I, Becker M, Lemm M, *et al.* Characterization of a new tumor in NMRI-mice suitable for chemotherapeutic experiments. *Arch Geschwulstforsch* 1987; **57**: 105–14.
20. Grove RB, Eckelmann WC, Reba RC. Distribution of labelled Bleomycin in normal and tumor-bearing mice. *J Nucl Med* 1973; **14**: 917–9.
21. Bache K, Kuhn B, Troldenier H. Bestimmung pharmakokinetischer Grössen in der Veterinärmedizin mittels eines EDV-Programms. *Arch Exper Vet Med* 1985; **39**: 296–302.
22. Scherphof G, Roerding F, Dijkstra J, *et al.* Uptake of liposomes by rat and mouse hepatocytes and kupffer cells. *Biol Cell* 1983; **47**: 47–54.
23. Gutteridge JMC, Shute DJ. Iron-dioxygen-dependent changes to the biological activities of Bleomycin. *J Inorg Biochem* 1981; **15**: 349–57.
24. Kono A, Matsushima Y, Kojima M, *et al.* Cobalt chelate of bleomycin. I. Pharmacochemical properties and distribution in tumor bearing mice. *Chem Pharm Bull (Tokyo)* 1977; **25**: 1725–31.
25. Alberts DS, Cheng NS, Woolfenden JM, *et al.* Pharmacokinetics of Bleomycin in man. III. Bleomycin-^{57}Co vs Bleomycin. *Cancer Chemother Pharmacol* 1979; **3**: 33–40.
26. Kono A, Kojima N, Maeda T. The tumor specific localizing agent for adioisotope image. The preparation of labelled bleomycins and their distribution in the tumor bearing mice. *Radioisotopes* 1972; **21**: 118–20.

27. Vos CM, Westra G. In the structure of the Co–Bleomycin A complex. *J Labelled Comp Radiopharmaceut* 1980; **XVIII**: 173.
28. Kahn PC, Milunsky C, Dewanjee MK, *et al.* The place of Co–Bleomycin scanning in the evaluation of tumors. *Am J Roentgenol Radium Ther Nucl Med* 1977; **129**: 267–72.
29. Rasker JJ, Van de Poll MA, Beekhuis H, *et al.* Some experience with Co-labelled bleomycin as a tumor-seeking agent. *J Nucl Med* 1975; **16**: 1058–61.
30. Schmilowski A. *Experiences with ^{57}Co-labelled Bleomycin in Tumor Diagnosis.* Springfield, IL: National Technical Information Service 1980; INIS-mf-5271.
31. Fujimoto S, Ogawa M. Antitumor activity of Mitoxantron against murine experimental tumors: comparative analysis against various antitumor antibiotics. *Cancer Chemother Pharmacol* 1982; **8**: 157–62.
32. Goldin AA, Kline I. The bleomycins experimental tumor activity. In: Carler SK, Crooke ST, Umezawa H, eds. *Mechanism of Bleomycin: Current Status and New Developments.* New York: Academic Press, 1978: 91–106.
33. Uehara Y, Hori M, Umezawa H. Specificity of transport of Bleomycin and cobalt-Bleomycin in L5178 Y cells. *Biochem Biophys Res Commun* 1982; **104**: 416–21.
34. Antholine WE, Petering DH, Saryan LA, *et al.* Interactions among iron (II) bleomycin, Lewis bases, and DNA. *Proc Natl Acad Sci USA* 1981; **78**: 7517–20.
35. Burger RM, Peisach J, Horwitz SB. Mechanism of Bleomycin action: *in vitro* studies. *Life Sci* 1981; **28**: 715–27.
36. Umezawa H, Takita T. Nucleotide-sequence-specific binding and cleavage of DNA by Bleomycin. *Tetrahedron* 1984; **40**: 501–9.
37. Takaita T, Umezawa H. Bleomycin and its derivatives. *Alfred Benzon Symp* 1984; **20**: 239–52.

(Received 18 September 1991; accepted 30 September 1991)