Cancer Chemother Pharmacol (1986) 18: 247–251

Cancer Chemotherapy and Pharmacology

Pharmacokinetics and tissue disposition of the biological response modifier BAY i 7433 (copovithane) in patients with cancer

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Summary. Copovithane is an uncharged, water-soluble, synthetic polymer with an average molecular weight of 5800 daltons. It demonstrates antitumor activity in vivo against a variety of tumors in animal models but is inactive in vitro. This agent has been found to have immunorestorative activity in man. In concert with its phase I clinical trial, copovithane concentrations were analyzed by HPLC in plasma, urine, and autopsy and in tumor biopsy specimens obtained from patients. Copovithane was cleared from plasma biphasically with a mean $t_{1/2}\alpha$ of 11.1 ± 4 min and a $t_{1/2}\beta$ of 246 ± 78 min at the dose of 1 g/m^2, while the plasma half-lives increased to 57.7 ± 12 and 718 ± 149 for the alpha and beta phases, respectively, at the 10 g/m^2 dose, demonstrating clear, dose-dependent pharmacokinetics. There were no significant differences between dose 1 and dose 4 pharmacokinetics. The apparent volume of distribution (V_d) was 14.5 ±1. at the 1 g/m^2 dose and increased to 73 l. at the 33 g/m^2 dose. The calculated mean clearance rate for copovithane in plasma was between 2.4 and 5.4 mg/kg × min and did not appear to be dose-dependent. The urinary excretion of copovithane was approximately 5% of the administered dose over 120 h at the 1 g/m^2 dose and decreased to 1% at the 33 g/m^2 dose. In seven tumor biopsy samples, concentrations of drug in tumor varied from 1- to 1000-fold higher than that found in concurrent plasma samples. In three autopsy samples, the highest concentrations were found in kidney, intestine, and liver, in decreasing order. These studies show that copovithane exhibits dose-dependent changes in pharmacokinetics at doses between 1 and 33 g/m^2. However, copovithane does penetrate well to tumor tissues, achieving high tumor/plasma ratios. In addition, copovithane concentrations were highest in kidney tissue, which may be a site for potential organ toxicity.

Introduction

Copovithane (BAY i 7433, Fig. 1) is a synthetic copolymer of 1,3 bis (methylaminocarboxy)-2-methylene propane carbomate and *N*-vinylpyrrolidone, with an average molecular weight of 5800 daltons. This uncharged, high-molecular-weight polymer is highly soluble in aqueous solvents. In animal studies copovithane was found to be a well-tolerated compound, without significant toxicity but exhibiting antitumor activity in animal studies after a single injection either before or after inoculation of tumor cells [4]. Copovithane was found to have significant in vivo antitumor activity against a wide panel of tumors, including sarcoma 180, P388 leukemia, carcinoma F0771, and fibrosarcoma F1026, in mice, as well as activity against Walker 1098 tumor in rats.

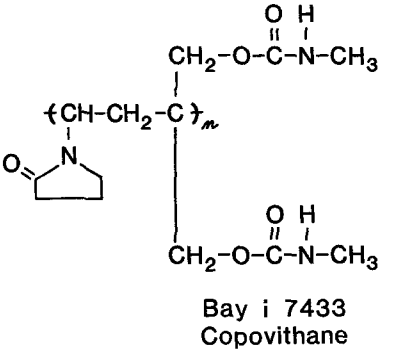

Fig. 1. Structure of copovithane

The mechanism of antitumor action of this agent has not yet been elucidated. However, copovithane is not cytostatic or cytotoxic in vitro, suggesting that this agent requires a metabolic transformation step in vivo or that it operates by way of modification of the host immunological responses to the tumor [4].

In concert with its phase I clinical trial, we developed a high-performance liquid chromatographic method for the analysis of copovithane in plasma, urine [3], and tissue samples and have applied this method to analysis of patient samples to determine the pharmacokinetics and tissue distribution of copovithane in man.

Materials and methods

All materials purchased from regular commercial suppliers were of reagent grade or higher. Distilled-in-glass acetonitrile was purchased from Burdick and Jackson Co.,

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Muskegon, Mich. All solvents were filtered, vacuum-degassed, and sparged with nitrogen immediately before use.

Chromatography. All analyses were performed with a Waters Associates (Milford, Mass) liquid chromatograph consisting of a model 710B sample processor, a model M6000 pump, a model 720 system controller, a data module and a model 450 variable-wavelength UV detector. An analytical reverse-phase (30 cm × 3.9 mm, 10 μm particle size) C-18 column from Waters Associates was used for all analyses. The mobile phase consisted of 30% acetonitrile and 70% water. The flow rate was 2 ml/min. The column eluate was monitored for UV absorbance at 340 nm.

Plasma and urine. Aliquots (2 ml) of plasma or urine containing copovithane were placed in 15-ml Corex test tubes and cooled on ice for 5 min. Plasma proteins were precipitated by the addition of 200 μl 10 *N* perchloric acid. The samples were vortexed vigorously and allowed to stand for 5 min on ice. The samples were spun at 17000 *g* for 15 min, after which the supernatants were transferred to glass (12 × 75 mm) test tubes and 200 μl 10 *N* KOH was added to neutralize each sample. The samples were again cooled on ice for 5 min and then centrifuged for 3 min in a Serofuge II centrifuge.

The supernatants were transferred to 15-ml Corex test tubes, and 2 ml hot (85 °C) saturated sodium chloride solution were added to each sample. The drug was then extracted three times by addition of 3 ml chloroform (Fisher Scientific Co. Fairlawn, NJ). The samples were vortexed vigorously and then spun at 17000 *g* for 15 min. The chloroform extracts were combined and then dried down under a nitrogen stream. The samples were then reconstituted with 1 ml 5 *N* hydrochloric acid, quantitatively transferred to a 3.5-ml screw-cap vial, sealed, and placed in a 160 °C oil bath. After 16 h the samples were removed, cooled on ice, and adjusted to neutrality with 1 ml 5 *N* sodium hydroxide. The pH of each sample was further adjusted to 8.0 by the addition of 3 ml 1 *M* sodium bicarbonate buffer, pH 8.0. An aliquot (0.75 ml) of a 0.5% solution of trinitrobenzene sulphonic acid (TNBS, Sigma Chemical Co., St. Louis, Mo.) in acetone was added. The samples were then incubated in the dark for 150 min and were extracted three times with 3 ml ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under a nitrogen stream. The samples were reconstituted in 250 μl methanol: H_2O (1 : 1) and chromatographed as described above.

Tissues. Tissue samples (0.5 g) were homogenized (Brinkman Polytron) in 2 ml distilled H_2O. Tissue homogenates were then processed as described for plasma and urine.

Patients. Patients with histologically proven malignancy were entered into the study. All patients gave informed consent in writing according to institutional guidelines. Patients received copovithane at doses between 1 and 33 g/m^2 in 100 ml 5% dextrose in water, administered i.v. over 15 min. Patients were treated once weekly for 10 weeks without dose esculation. Five different patients were entered at each dose level. Pharmacokinetic studies were performed after the first (week 1) and after the fourth (week 4) dose of copovithane. Blood samples of 11 ml were collected in tubes containing heparin at 0 (infusion end), 15, 30, 45, 60, 90, 120 min and at 4, 6, 8, 10, 24 and 36 h after drug administration. Urine was collected as voided in 24-h aliquots. Aliquots of plasma and urine were frozen until analysis by HPLC. Standard pharmacokinetic parameters were obtained by nonlinear regression analysis and an exponential stripping computer program.

Results

Standard curves for copovithane quantitation in plasma and urine were generated using the method of external standard addition. Standard curves for each particular tissue examined (except for tumor) were also generated by the method of external standard addition to tissue samples obtained from autopsy of patients who had not received copovithane therapy. Standard curves for copovithane in plasma, urine, and tissues were linear over the concentration range tested. Recovery rates for plasma and urine were approximately 40% and 43%, respectively. Recovery rates for tissues varied with the tissue type tested, but were between 10% and 40%. Inter- and intra-assay variability of standards was 19% and 2.3%, respectively. The functional lower limit of detection was approximately 15 μg/ml for plasma and 20 μg/ml for urine. The lower limit of detection for tissues varied between 10 and 15 μg/g tissue.

The plasma clearance curves for copovithane in patients who received one dose of either 1 g/m^2 or 10 g/m^2 are shown in Fig. 2. The clearance curves for both doses closely fitted an open two-compartment mathematical model. The half-lives for the alpha and beta phases were 11.1 ± 4.0 min and 246 ± 78 min, respectively, for the 1 g/m^2 dose and increased to 57.7 ± 12 min and 718 ± 149 min, respectively, for the 10 g/m^2 dose. The pharmacokinetic data of 48 patients studied who received copovithane in doses of between 1 and 33 g/m^2 are summarized in Table 1. There were no statistical differences (by Mann-Whitney or Chi-square) in the pharmacokinetics calculated for the first copovithane dose and for dose 4. As demonstrated in Fig. 2, however, the mean half-lives (both alpha and beta) did appear to increase with increasing dose level. Similarly, the calculated apparent volume of distribution (V_d) was not significantly different between

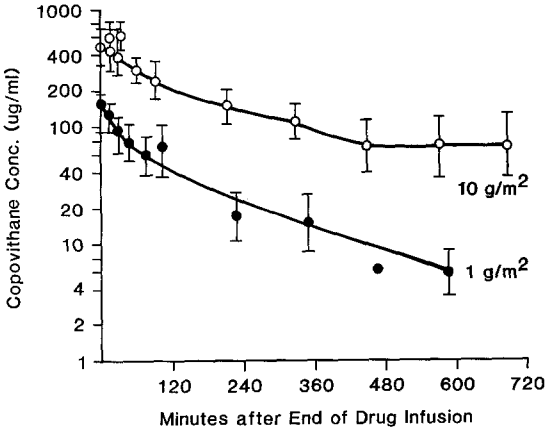

Fig. 2. Plasma clearance of copovithane from patients who received doses of either 1 g/m^2 (6 patients) or 10 g/m^2 (7 patients). Values shown are the means, while the *solid lines* represent the least-squares fit lines for the first drug dose at these levels

Table 1. Pharmacokinetics of copovithane[a]

Dose (g/m^2)	No. of Pts	Plasma $t_{1/2}$ (min)		V_d (l)	Cxt (mg/ml × min)	Clp (ml/kg × min)
		Alpha	Beta			
1.0	5	11.1± 4.0	246.0± 78	14.5± 4	14.0± 7.6	5.4±2.4
2.0	3	8.7± 4.4	55.0± 16	13.3± 4.6	17.7± 4.0	3.5±0.9
4.0	4	30.0± 8.5	147.0± 42	18.9± 4.7	48.0± 8.6	2.5±0.8
6.0	6	33.6±29.3	678.0±124*	20.5± 3.2	85.4± 4.2	2.8±0.8
10.0	4	57.7±12	718.0±149*	35.3±11*	133.0±66	4.5±2.4
15.0	5	86.2±20	145.7±132	45.0± 8.0*	151.0±23	2.4±0.4
33.0	2	125	373	73	176	4.2

[a] Values shown are means ± SEM and were obtained following the first dose of drug
[b] Abbreviations used are: $t_{1/2}$, half-life; V_d, apparent volume of distribution; Cxt, concentration times time; Clp, clearance rate from plasma
* Statistically significant difference ($P<0.001$) from result with 1 g/m^2 dose

the first dose and the fourth at the same dose level; however, the V_d did show a statistically significant increase with increasing dose, as shown in Table 1.

The clearance rate of copovithane from plasma (Clp) was not significantly affected by the number of doses at the same dose level (data not shown), nor did increasing dose levels affect the clearance rate (Clp, Table 1). The urinary excretion of copovithane in patients who received either 1 or 10 g/m^2 is shown in Fig. 3. Most of the drug (approximately 3%–5%) was found to be excreted within 24 h after administration. However, as shown in Table 2, with

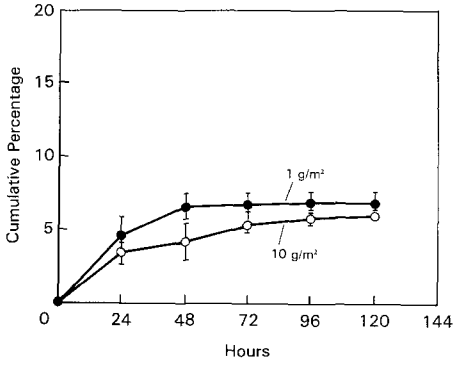

Fig. 3. Cumulative urinary excretion of patients who received copovithane doses of either 1 g/m^2 (6 patients, *closed circles*) or 10 g/m^2 (7 patients, *open circles*). Values are for the first dose only at each level

Table 2. Urinary excretion of copovithane[a]

Dose (g/ml^2)	No. of Pts	Total urinary recovery (% of dose in 120 h)	Total drug excreted (g) over 120 h
1	4	5.2±1.6	0.08
2	3	5.4±2.2	0.15
4	4	3.4±1.3	0.20
6	4	5.1±2.4	0.50
10	4	4.1±1.7	0.60
15	5	1.9±0.8	0.53
20	4	1.0±0.3	0.34
33	2	1.0±0.2	0.45

[a] Values shown are means ± SEM

increasing dose a decreasing percentage of the drug appeared to be excreted in the urine. Analysis of the total amount of drug excreted (Table 2) showed that increasing the drug dose from 1 to 6 g/m^2 resulted in an increase in the total amount excreted (from 0.075 g at the 1 g/m^2 dose to 0.5 g at the 6 g/m^2 dose) over 120 h. Thereafter, with increasing dose, the total amount of drug excreted in urine remained between 0.6 and 0.45 g.

The concentration of copovithane was determined in biopsy samples obtained from six patients with metastatic melanoma and one patient with a leiomyosarcoma (Table 3). Copovithane was also measured in plasma samples obtained at the time of biopsy. As shown in Table 3, tumor concentrations of copovithane in all tumors studied far exceeded the concentration of drug in concurrent plas-

Table 3. Analysis of copovithane in tumor biopsy samples and in concurrent plasma samples

Pt No.	Tumor type	No. of doses	Total dose prior to biopsy (g)	Plasma concentration (μg/ml)	Tumor concentration (μg/g)	Tissue to plasma ratio
1	Melanoma	25	394	3.5	228	65.1
2	Leiomyosarcoma	5	260	23.2	2558	110
3	Melanoma	5	260	2.4	703	293
4	Melanoma	15	885	LL	648.2	–
5	Melanoma	1	11	0.54	570.1	1056
6	Melanoma	10	100	LL	12.6	–
7	Melanoma	15	150	ND	39.1	–

ND, not determined; LL, below assay lower limit of detection

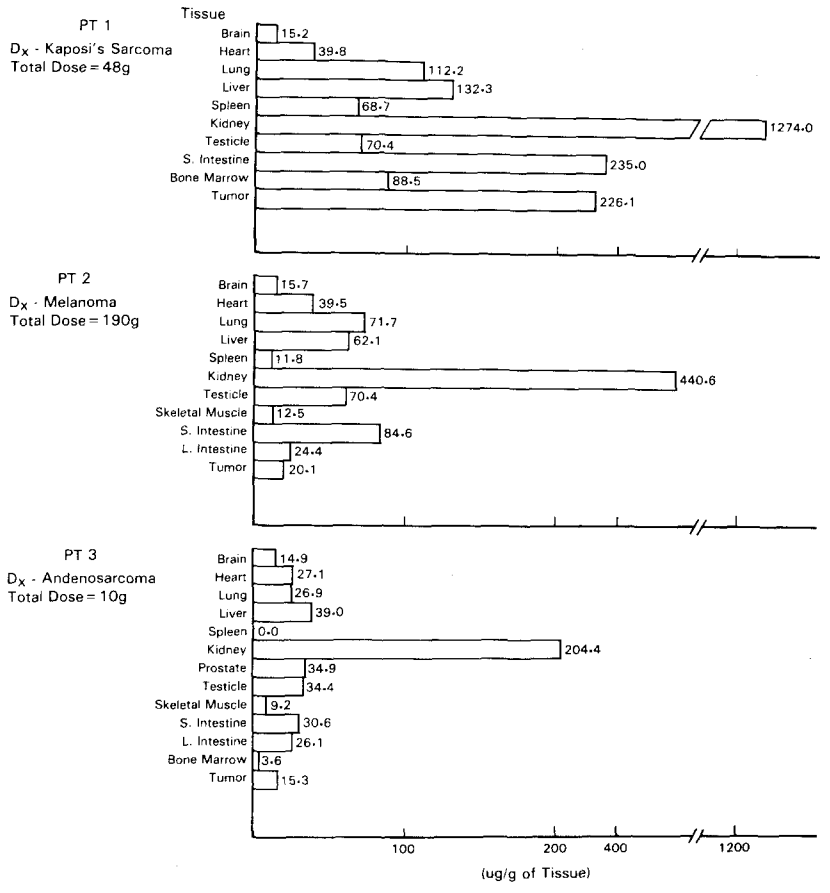

Fig. 4. Tissue distribution of copovithane in autopsy samples from patients with solid tumors. Patient 1 received copovithane 8 g/m^2 weekly for 4 weeks (total dose 48 g). Patient 2 received copovithane 22 g/m^2 weekyl for 6 weeks (total dose 190 g). Patient 3 received copovithane 6 g/m^2 once (total dose 10 g)

ma samples. This clearly demonstrates that the agent can significantly distribute to and accumulate within tumor sites. The disposition of copovithane in autopsy samples obtained from three patients treated with various doses of copovithane is shown in Fig. 4. Of the patients studied, three died and were subsequently autopsied between 4 and 23 days after the last drug dose. In all three, the kidney tissue showed by far the highest concentration of agent, despite the poor excretion of copovithane in urine. Significant amounts of drug were also found in lung, liver, and small intestine. In one patient with Kaposi's sarcoma (Pt 1), copovithane levels in tumor tissue (226 μg/g) were almost 10-fold higher than those found in one patient with adenosarcoma (15.3 μg/g) or one with melanoma (20.1 μg/g). Similar amounts of copovithane were found in normal brain tissue (approximately 15 μg/g) of all three patients, demonstrating that this agent can cross the blood-brain barrier.

Discussion

These studies show that the pharmacokinetics of copovithane can be subtantially modified with increasing dose. Although the total clearance rate for the agent appears to be independent of dose both the plasma half-lives and the apparent volume of distribution increase with increasing dose. The increase in plasma-half-life with increased dose has previously been observed with other therapeutic agents [2] and may be partly explained by saturation of clearance or metabolic mechanisms at the higher doses. As shown in Table 2, the increase in plasma $t_{1/2}$ may be due, in part, to saturation of renal clearance mechanisms. Clearance of copovithane by the kidney does not exceed 600 mg/120 h and it appeared to be inhibited at copovithane doses above 10 g/m^2. However, the underlying events which lead to increased apparent volume of distribution with increasing dose are not readily apparent. This may suggest that higher doses of copovithane may cause distribution to previously cloistered tissue sites. Alternatively, copovithane, like other agents [1], may be responsible for increasing its own metabolism at higher doses, thereby changing its apparent volume of distribution.

Tissue disposition studies of copovithane in tumor biopsy samples demonstrate that this agent penetrates well to tumor sites, achieving a high tissue-to-plasma ratio with repeated drug administration. The highest drug concentrations were found in normal kidney tissue, suggesting that this may be a potential site for toxicity. However, since in vitro studies have shown that copovithane dose not have a direct antigrowth effect [4], it is not clear whether high concentrations of drug in organs such as kidney or in tumor sites will have a beneficial or a detrimental effect. Furthermore, since the tissue target for copovithane is not known, it is not clear whether accumulated tissue disposition of copovithane, which apparently occurs after multiple doses, has a beneficial antitumor effect.

The analytical procedure utilized for analysis of copovithane in plasma, tissue, and urine cannot distinguish between parent drug and possible metabolites. However, preliminary in vitro studies with copovithane have demonstrated that the agent remains relatively intact after incubation with tissue sections, urine, or tumor cells in vitro

(M. G. Rosenblum et al., 1984 unpubl. observation). Nevertheless, this does not dismiss the possibility that copovithane may be extensively metabolized in vivo. Further studies are under way to develop analytical procedures addressing these questions. Until further studies can clarify the biochemical mechanism of action or the possible effector site of copovithane, it is difficult to make specific recommendations regarding clinical studies with copovithane based upon the pharmacokinetic and tissue disposition studies presented herein. Nevertheless, we can clearly state that copovithane is cleared from the plasma biphasically and that the pharmacokinetics of clearance appear to be dependent on the dose administered. Renal clearance mechanisms, which are apparently saturable at copovithane doses above 10 g/m^2, can account for a maximum of only 600 μg drug excreted in urine over 120 h. In addition, copovithane appears to distribute well to normal tissues, the kidney being the primary site of distribution. Finally, after repeated administration, copovithane appears to accumulate well in tumor tissues.

Acknowledgements. The authors wish to express their sincere appreciation to Debra Frye, Kelly Campbell and Karen Hagberg for coordinating patient studies and sample acquisition. In addition, the authors wish to thank both Lawrence Cheung for his superb technical assistance in HPLC analysis and data analysis and Lydia Morris for assistance in typing this manuscript.

References

1. Conney A (1971) Environmental factors influencing drug metabolism: In: Fundamentals of drug metabolism and drug disposition. Waverly, Baltimore P 253
2. Gutterman JU, Rosenblum MG, Rios A, Fritsche HA, Quesada JR (1984) Pharmacokinetic study of partially pure gamma-interferon in cancer patients. Cancer Res 44: 4164
3. Rosenblum MG, Hortobagyi GN, Wingender W, Hersh EM (1984) Analysis of the antitumor agent BAYi 7433 (copovithane) in plasma and urine by high performance liquid chromatography. J Liquid Chromatogr 7: 159
4. Schlumberger HD (1981) BAYi 7433: A synthetic polymer with antitumor activity. In: Augmenting agents in cancer therapy. Raven, New York, p 373

Received August 5, 1985/Accepted July 21, 1986