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Distribution of the novel anticancer drug candidate Brequinar Sodium (DuP 785, NSC 368390) into normal and tumor tissues of nude mice bearing human colon carcinoma xenografts

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Summary. The distribution of the novel anticancer drug candidate Brequinar Sodium (DuP 785, NSC 368390) was studied in control mice and mice implanted subcutaneously with human colon carcinoma xenografts. Mice were given radiolabeled ^{14}C-Brequinar Sodium intravenously. Brequinar concentrations in blood and various tissues were determined at 1, 6, and 24 h after drug administration. Within 1 h Brequinar distributed to the tumor and all other tissues studied. The tumor-to-blood drug concentration ratios ranged from 0.19 to 0.41. Radioactivity in the liver and small intestine at 1 h accounted for 17% and 13%, respectively, of the dose given. Elimination rates of Brequinar from all tissues were approximately equal to that from blood. Comparison of blood concentrations determined by both radioactivity and HPLC methods suggests that the intact drug is probably the only form in the blood.

Introduction

The novel anticancer drug candidate Brequinar Sodium (DuP 785, NSC 368390), 6-fluoro-2-(2'-fluoro-1,1'-biphenyl-4-yl)-3-methyl-4-quinoline-carboxylic acid sodium salt, has demonstrated good activity against a number of experimental tumors, including several human colon carcinoma xenografts in nude mice [4]. The compound has been shown to produce its antitumor effect by inhibiting the de novo pyrimidine enzyme dihydroorotate dehydrogenase [1]. Because of its activity against human solid tumors in nude mice, Brequinar Sodium has been entered into clinical trials. We have now studied the distribution of radiolabeled compound into normal and tumor tissues of nude mice bearing human colon carcinoma xenografts. This information would also indicate (a) whether Brequinar uptake into solid tumors correlates with its anticancer efficacy and (b) whether the drug candidate distributes to various tissues that are sites of metastatic deposits of solid tumors.

Materials and methods

Drug and chemicals. Brequinar Sodium was synthesized by the Medicinal Chemistry Section, DuPont Pharmaceuticals, and the ^{14}C-labeled compound [2-^{14}C DuP 785] was prepared by DuPont NEN Research Products. The specific activity of the radiolabeled compound was 7.55 mCi/mmol. DuP 416, the internal standard, is a diarylpyrrole synthesized by DuPont Pharmaceuticals. Dichloromethane, acetonitrile, and phosphoric acid were HPLC grade. All other chemicals were reagent grade.

Tumor cells and animals. Clone A human colon cancer cells were isolated from the heterogeneous DLD-1 colon tumor line established from a surgical specimen of primary colon adenocarcinoma [2]. Clone A cells were grown in 100-mm tissue culture dishes (Falcon Plastics, Oxnard, Calif) in RPMI-C as previously reported [3]. The mice used in these experiments were outbred Swiss mice bearing the nude (nu/nu) gene [5, 6]. Mice weighing 22–25 g were inoculated with 1×10^7 cultured clone A cells subcutaneously in the flank region. Tumors were allowed to grow for 3–4 weeks to a size of about 1 g. The mice in the control and tumor-bearing groups were pair-matched for their body weights.

Experimental design. Radiolabeled Brequinar Sodium (approximately 50 mg/kg) was given via tail vein to tumor-bearing and control mice. Each mouse received 0.3 ml of the dosing solution (5.42 μCi), which contained 5 mg/ml Brequinar Sodium in 0.9% NaCl solution. At 1, 6, 12, and 24 h after compound administration, four mice from each of the tumor-bearing and control groups were sacrificed to collect blood, tumor, and 19 other tissues. One to three portions (approximately 100 mg) of each tissue were dissected for radioactivity measurement. All samples were stored frozen at −20° C until analysis. The gastrointestinal contents were not removed in the first experiment. In a second experiment, blood and the gastrointestinal tract were collected from control mice only and the gastrointestinal contents were removed. Radioactivity in the blood and tissues was measured and concentrations of intact Brequinar in blood were also determined.

A Packard Oxidizer (Model 306) was used to completely oxidize the tissues. The resulting ^{14}C-carbon dioxide was trapped and collected in a mixture of 6 ml Carbo-Sorb and 9 ml Permafluor (Packard). The radioactivity was then analyzed by a Packard Scintillation Counter (Model 4640 TRI-CARB). Counting efficiency was determined by the external standard ratio method and a quench-correction curve.

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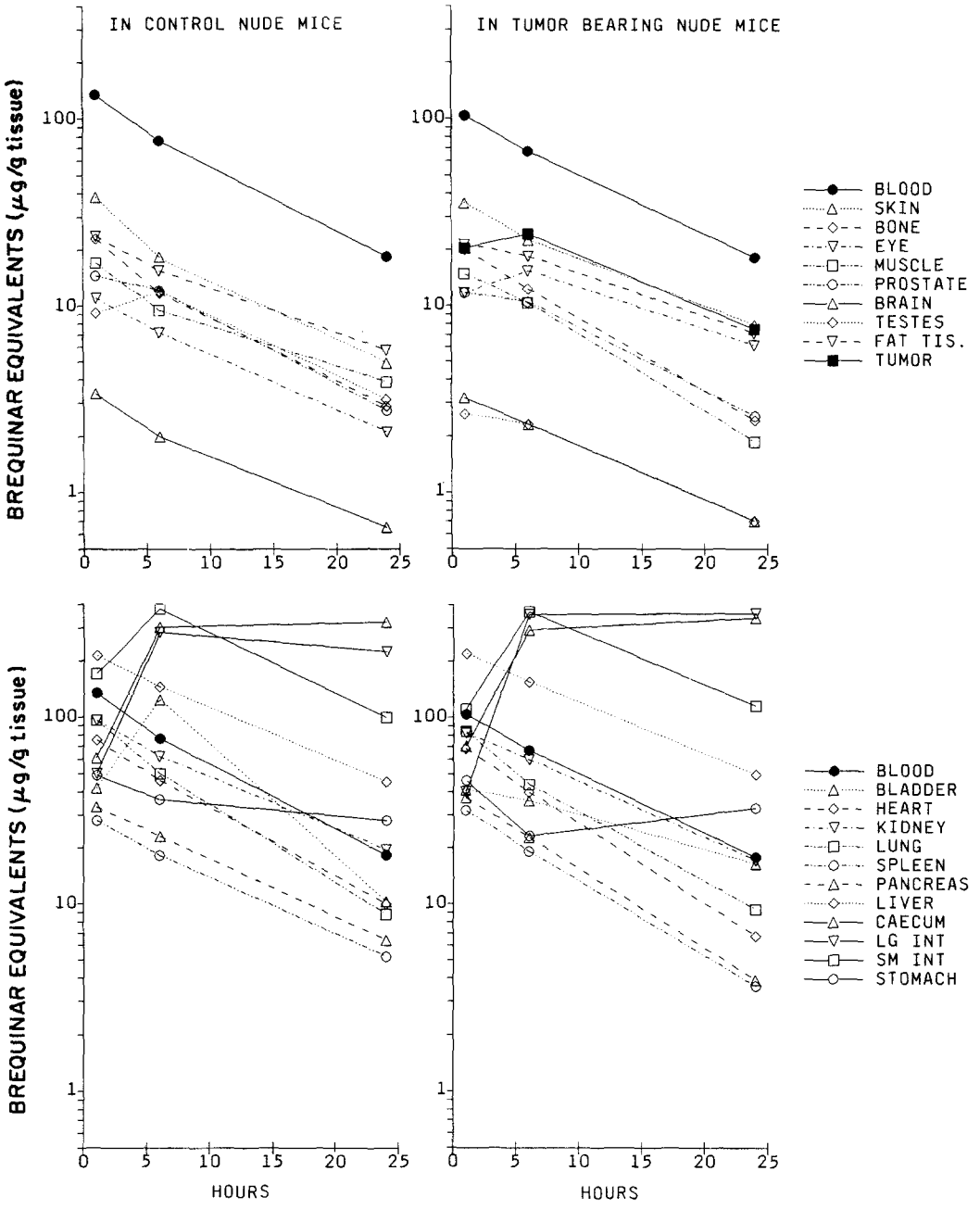

Fig. 1. Tissue levels of Brequinar equivalent in control and tumor-bearing mice given 50 mg/kg i.v. dose of ^{14}C-labeled Brequinar Sodium. Gastrointestinal contents were not removed

Brequinar blood concentrations were determined by high-performance liquid chromatography (HPLC). Aliquots of 100 µl were extracted with 5 ml dichloromethane in the presence of 200 µl tetrabutylammonium hydroxide (4 m*M*) as the ion-pairing agent and 1.25 µg DuP 416 as the internal standard. The dichloromethane layer was transferred to another tube and evaporated to dryness. The residue was reconstituted with the mobile phase (63:37 acetonitrile:0.045 *M* phosphoric acid) and injected onto the HPLC. The HPLC system was equipped with a Zorbax TMS column and a UV detector (Waters Model 441) at 254 nm. The flow rate of the mobile phase was 1 ml/min.

Results

Figure 1 shows the tissue levels of Brequinar equivalent after an i.v. dose was given to tumor-bearing and control mice (the first experiment). Since drug levels in tissues were measured by radioactivity, the values (expressed as Brequinar equivalent) shown in this figure were the sum of the concentrations of Brequinar and any metabolites. Brequinar was widely distributed through all the tissues. The presence of tumor cells in the flank region did not change the distribution pattern. The elimination rates of Brequinar in all tissues were similar except in the stomach, cecum, and large intestine. Radioactivity in these three tissues appeared to remain at constant levels between 6 and 24 h.

The tissue-to-blood Brequinar concentration ratios are shown in Table 1. The tumor-to-blood ratio ranged from 0.20 to 0.45 and the liver-to-blood ratio ranged from 1.61 to 2.76. Very little compound reached the brain, as the ratio was only between 0.02 and 0.04. Table 2 shows the per-

Table 1. Mean tissue-to-blood ratios in control and tumor-bearing mice given a ^{14}C-Brequinar Sodium i.v. dose ($n = 4$)

Tissue	Control mice			Tumor-bearing mice		
	1 h	6 h	24 h	1 h	6 h	24 h
Tumor	NA[a]	NA	NA	0.20	0.35	0.45
Blood	1.00	1.00	1.00	1.00	1.00	1.00
Cecum	0.45	3.92	18.70	0.67	4.40	20.27
Liver	1.61	1.92	2.76	2.15	2.32	2.73
Large intestine	0.38	3.84	12.56	0.39	5.34	20.97
Small intestine	1.31	5.24	5.08	1.10	5.56	6.66
Skin	0.29	0.24	0.27	0.35	0.34	0.35
Stomach	0.37	0.53	1.91	0.45	0.35	2.07
Bladder	0.31	1.59	0.69	0.41	0.54	0.96
Bone	0.17	0.15	0.16	0.20	0.18	0.13
Eye	0.08	0.09	0.12	0.12	0.21	0.38
Fat tissue	0.18	0.20	0.33	0.21	0.27	0.32
Heart	0.57	0.59	0.54	0.65	0.58	0.38
Kidney	0.73	0.80	1.09	0.80	0.88	0.99
Lung	0.71	0.65	0.50	0.83	0.65	0.48
Muscle	0.13	0.12	0.19	0.15	0.15	0.10
Pancreas	0.24	0.30	0.35	0.36	0.33	0.22
Prostate	0.11	0.15	0.15	0.11	0.16	0.13
Brain	0.02	0.03	0.04	0.03	0.04	0.04
Spleen	0.20	0.24	0.29	0.31	0.28	0.19
Testes	0.07	0.16	0.18	0.13	0.17	0.17

[a] NA, Not applicable

Table 2. Mean percentage of ^{14}C-Brequinar Sodium dose distributed in each tissue of the control and tumor-bearing mice given an i.v. dose ($n = 4$)

Tissue	Control mice			Tumor-bearing mice		
	1 h	6 h	24 h	1 h	6 h	24 h
Tumor	NA[a]	NA	NA	0.84	1.14	0.47
Blood	18.76	10.12	2.50	16.75	9.58	2.66
Cecum	1.13	7.65	10.48	1.52	7.42	8.58
Liver	17.34	12.08	3.72	21.52	13.21	4.64
Large intestine	1.00	6.69	4.60	0.80	10.26	9.87
Small intestine	12.59	25.77	6.64	9.49	25.91	8.53
Skin	6.49	2.96	0.82	6.96	3.95	1.38
Stomach	0.94	0.81	0.61	0.82	0.40	0.59
Bladder	0.11	0.32	0.02	0.10	0.07	0.03
Bone	4.54	2.21	0.47	4.54	2.48	0.50
Eye	0.03	0.02	0.01	0.03	0.05	0.02
Fat tissue	2.33	1.47	0.57	2.42	1.87	0.72
Heart	0.77	0.47	0.11	0.79	0.36	0.06
Kidney	2.63	1.62	0.50	2.58	1.49	0.47
Lung	0.88	0.53	0.10	1.18	0.45	0.10
Muscle	8.31	4.46	1.85	8.32	5.21	0.95
Pancreas	0.23	0.15	0.04	0.25	0.13	0.03
Prostate	1.03	0.15	0.03	0.15	0.13	0.05
Brain	0.08	0.07	0.02	0.08	0.06	0.02
Spleen	0.21	0.09	0.03	0.26	0.12	0.03
Testes	0.12	0.13	0.04	0.18	0.15	0.04
SUM	79.52	77.77	33.16	79.58	84.44	39.74

[a] NA, Not applicable

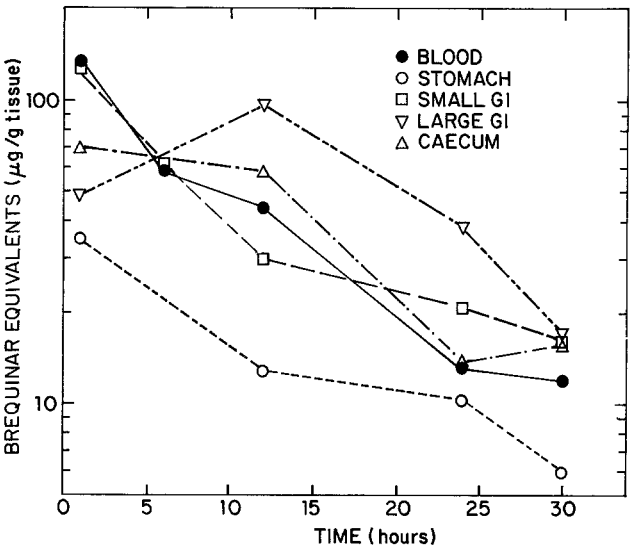

Fig. 2. Tissue levels of Brequinar equivalent in control mice given 50 mg/kg i.v. dose of ^{14}C-labeled Brequinar Sodium. Gastrointestinal contents were removed

Table 3. Brequinar blood concentrations (μg/ml) in control mice given an i.v. dose of ^{14}C-Brequinar. Concentrations were measured by both radioactivity and an HPLC method

Time (h)	Mouse number	Brequinar concentration by radioactivity	Brequinar concentration by HPLC
1	1	148.00	153.49
	2	125.40	142.00
	3	110.60	131.55
	4	152.55	161.80
	Mean	134.15	147.21
6	5	47.40	48.65
	6	53.90	77.17
	7	69.25	65.50
	8	63.25	64.27
	Mean	58.45	63.90
12	9	49.95	48.92
	10	35.60	37.08
	11	39.95	43.49
	12	52.05	43.62
	Mean	44.40	43.28
24	13	9.10	7.83
	14	10.75	9.66
	15	14.35	13.27
	16	18.45	17.41
	Mean	13.15	12.04
30	17	12.95	12.21
	18	17.80	15.41
	19	7.45	7.82
	20	9.80	10.38
	Mean	12.00	11.46

centage of the i.v. dose that was found in each tissue. Because the total weights of the blood, skin, bone, fatty tissue, and muscle cannot be measured directly, they were estimated as 7%, 9%, 10%, 5%, and 25%, respectively, of the total body weight (JC Gaylord, personal communication). Radioactivity in the blood, liver, and small intestine at 1 h accounted for 19%, 17%, and 13%, respectively, of the dose given.

The constant drug levels observed for 24 h in the stomach, cecum, and large intestine in the first experiment may

result from the gastrointestinal contents. To investigate this, drug distribution in the gastrointestinal tissues was further studied in control mice whose gastrointestinal contents were removed. The results of the second experiment showed that the drug elimination from these tissues paralleled that from the blood (Fig. 2). Drug concentrations in these tissues were also lower than those observed in the first experiment. Blood elimination half-lives of Brequinar in these two experiments were 8.16 and 7.24 h in control mice and 9.14 h in tumor-bearing mice.

Since radioactivity measurement does not differentiate the intact drug from metabolites, a specific HPLC method was used to determine the intact Brequinar concentrations. Blood concentrations of intact Brequinar in the second experiment were determined by the HPLC method. Table 3 compares the concentrations measured by total radioactivity and HPLC. The excellent agreement between the values obtained from these two methods suggests that the radioactivity in the blood is the intact Brequinar.

Discussion

The major finding of this study is that within 1 h following i.v. administration to mice, Brequinar distributed to a subcutaneously implanted human colon tumor xenograft and to all other tissues studied. Brequinar levels in epithelial tissues, including the colon carcinoma, ranged from 30% to 300% of those in blood. Only the brain and testes had lower concentrations (< 5% of blood levels). The elimination half-life of Brequinar from all tissues studied was approximately equal to the $t_{1/2}$ in blood of 7–9 h. This elimination $t_{1/2}$ agreed quite well with the plasma $t_{1/2}$ previously determined using nonlabeled compound and an HPLC method [7].

A previous study using CDF_1 mice showed that the majority of the compound was excreted in feces. Therefore, in the first experiment, in which gastrointestinal contents were included, drug levels in the gastrointestinal tissues were relatively higher than in the blood. The contents also contributed to the sustained high drug levels. When the contents were removed, as in the second experiment, the drug levels in these tissues were not as high as in the first experiment. In addition, the elimination rates in these tissues were similar to that in the blood. These results suggest that during multiple dosing Brequinar should not accumulate more in any tissue than in the blood.

Because radioactivity was measured to determine drug levels in tissues, we could not differentiate the intact drug from any of its metabolites. However, the similar blood concentrations obtained in the second experiment using both radioactivity and an HPLC method indicate that intact Brequinar is most likely the only form in the blood. Therefore, it is likely that intact Brequinar is also the only form in the nonmetabolizing tissues. The concentration of Brequinar in the colon tumor xenograft was approximately 30% of that in the blood; it is clear that Brequinar not only reaches the tumor, but is also taken up quickly by the neoplasm. This level of intact drug (> 10 μg/g tumor) is sufficient act against a spectrum of experimental murine and human solid tumors [1, 4].

Since most cancer patients succumb to metastases following surgical resection of the primary tumor, the need for an agent that has activity against disseminated tumors in an adjuvant setting is well known. Thus, it is important that an anticancer drug distribute to organs such as the liver and lung that are preferential sites of metastasis for many of the major carcinomas; Brequinar has such a distribution profile. The level of drug in liver was approximately twice that in blood over the 24-h time period studied. This finding could be important if Brequinar is used against disseminated colon cancer, since many colorectal cancer patients die of liver metastases [8]. In summary, as a novel drug candidate for the treatment of carcinomas and sarcomas, Brequinar distributes rapidly to tumor tissue and to potential metastatic sites.

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