Tissue Distribution of Copper-Labeled 3-Ethoxy-2-oxobutyraldehyde bis (Thiosemicarbazone) (Cu-64 KTS) in Mice and Rats: Concise Communication

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The antitumor activity of, 3-ethoxy-2-oxobutyraldehyde bis (thiosemicarbazone) (KTS), is related to the presence of copper(II) ion. We have studied the tissue distribution of Cu-64-labeled KTS in rats and mice carrying transplanted tumors to evaluate whether the uptake of the radioactivity in the tumor is adequate to warrant further investigation of the tracer as a tumor-seeking agent in patients.

Four groups of three or four animals each were studied: (a) mice with fibrosarcoma; (b) mice with mammary adenocarcinoma; (c) rats with fibrosarcoma; and (d) rats with squamous cell carcinoma of the lung. The animals were killed at intervals of 0.25, 1, 4, 24, and 48 hr after i.v. injection of 1.6×10^{-3} *M* Cu·KTS containing 3 to 18 μCi Cu-64. Blood, tumor, and six to ten additional tissues were counted for radioactivity.

The mouse fibrosarcoma concentrated Cu-64, reaching 15% of the administered dose/g at 48 hr after injection. This suggests that for tumor scanning, the 61.7-hr Cu-67 might be more suitable as a label for KTS than the 12.7-hr Cu-64.

J Nucl Med 21: 67–70, 1980

French and Friedlander (*1*) screened a number of thiosemicarbazones for antineoplastic activity against animal tumors. The antitumor activity of one of these compounds, 3-ethoxy-2-oxobutyraldehyde bis (thiosemicarbazone) (KTS) (Fig. 1) was shown by Petering and Buskirk (*2,3*) to require the presence of copper(II) ion.

The physicochemical properties of KTS and of KTS complexed with Cu^{2+} have been investigated by Petering (*4,5*), but tissue distribution has not been studied either in normal animals or in animals bearing transplanted tumors.

We studied Cu-64 KTS for its potential tumor-seeking properties in mice and rats transplanted with solid tumors. We report our findings in four groups of animals: (a) mice with fibrosarcoma; (b) mice with mammary adenocarcinoma; (c) rats with fibrosarcoma; and (d) rats

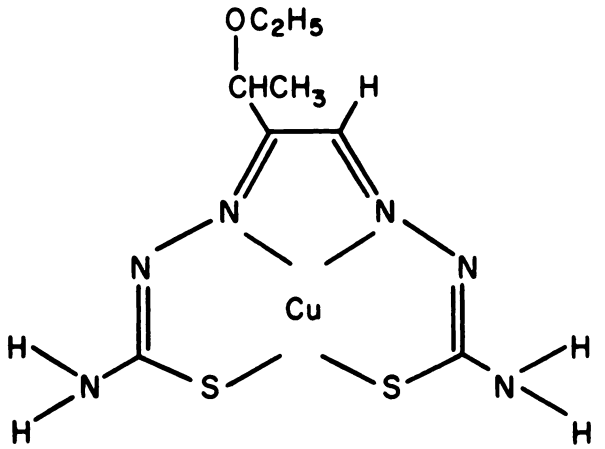

FIG. 1. Structure of 3-ethoxy-2-oxo-butyraldehyde bis (thiosemicarbazone).

Received Jan. 23, 1979; revision accepted May 30, 1979.

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with squamous cell carcinoma of the lung.

MATERIALS AND METHODS

KTS was obtained commercially* and was recrystallized from 95% (v/v) ethanol before use. BDF_1 female mice, 4–6 wk old (15–18 g), and female Fischer rats, 4–6 wk old (~125 g), were supplied by the Animal Care Center. The following animal tumors were kindly supplied by other laboratories: mouse mammary adenocarcinoma, MTG-B;† mouse fibrosarcoma, SAD_2;‡ rat fibrosarcoma, CSE;‖ and a squamous cell carcinoma transplantable in Fischer rats.§

A suspension of fresh tumor cells was prepared for transplantation with a Snell cytosieve (*6*) and ~0.1–0.2 ml injected subcutaneously into the flank of each animal. When the transplanted tumor was of visible size, the animals, under ether anesthesia, were injected by tail vein with 50–100 μl of 1.6×10^{-3} *M* Cu-64 KTS containing 3–6 μCi Cu-64 and ~5 μg of stable copper. A

TABLE 1. DISTRIBUTION OF RADIOACTIVITY FOLLOWING I.V. ADMINISTRATION OF Cu-64 KTS IN BDF_1 MICE; (% DOSE/g, MEAN AND RANGE)

No. of mice	Time (hr)	Blood	Fat	Heart	Kidney	Liver	Lung	Muscle	Tumor
				Adenocarcinoma					
3	0.25	6.4 (3.5–9.0)	0.9 (0.4–1.5)	33.9 (17.5–47.5)	16.6 (12.4–19.4)	12.4 (10.8–13.6)	121 (87.8–165.9)	3.5 (2.0–5.4)	1.9 (0.9–3.1)
3	1.0	2.4 (2.3–2.6)	—	17.0 (16.2–17.9)	12.5 (11.2–13.5)	35.7 (32.5–39.1)	44.7 (40.5–47.7)	2.1 (2.0–2.4)	3.8 (2.8–4.6)
3	4.0	2.7 (2.0–4.2)	0.4 (0.2–0.6)	16.5 (14.5–19.2)	7.2 (4.9–8.7)	42.2 (28.0–55.6)	29.5 (23.5–38.9)	1.3 (0.8–1.9)	6.0 (4.6–6.9)
3	24.0	2.4 (1.7–3.8)	0.2 (0.02–0.4)	10.3 (8.9–13.0)	7.1 (5.6–9.5)	13.6 (11.4–16.2)	15.1 (10.0–18.7)	0.8 (0.5–1.1)	3.2 (2.8–3.9)
				Fibrosarcoma					
3	0.25	5.8 (5.4–6.4)	1.1 (0.9–1.2)	22.9 (10.2–29.8)	12.4 (8.7–16.3)	15.8 (11.5–18.3)	82.3 (59.2–115.3)	4.6 (3.6–7.9)	4.6 (3.6–6.2)
3	1.0	2.5 (1.8–3.2)	1.1 (0.6–2.6)	15.9 (13.5–19.3)	10.0 (7.6–13.4)	16.3 (11.3–21.2)	28.0 (20.6–37.7)	2.7 (2.4–3.7)	3.5 (1.4–6.1)
3	4.0	2.1 (1.6–3.0)	0.9 (0.7–1.2)	13.2 (11.6–14.4)	5.9 (4.3–7.2)	30.4 (22.3–44.1)	13.9 (11.7–15.7)	1.1 (0.9–1.3)	7.9 (7.3–8.3)
3	24.0	2.0 (1.7–2.3)	1.2 (0.7–1.7)	9.0 (7.4–11.0)	4.8 (4.5–5.2)	9.7 (6.9–11.1)	8.5 (6.1–10.3)	0.7 (0.6–0.8)	10.1 (9.1–10.7)
3	48.0	1.4 (1.2–1.7)	0.4 (0.1–1.4)	8.4 (7.8–9.2)	6.5 (5.8–6.8)	11.6 (9.6–12.4)	8.3 (7.3–10.2)	0.7 (0.5–0.9)	15.4 (12.5–20.8)
				Fibrosarcoma—$^{64}CuCl_2$ Controls					
2	0.25	1.8 (0.3–3.4)	0.5 (0.2–1.2)	3.0 (2.8–3.5)	8.8 (8.2–9.6)	84.1 (77.6–92.3)	4.5 (3.2–6.0)	0.6 (0.6–0.7)	2.1 (1.3–2.9)
2	1.0	2.9 —	0.3 (0.3–0.4)	2.9 (2.5–3.2)	7.7 (6.3–9.0)	63.1 (60.6–62.8)	5.0 (4.7–5.2)	0.4 (0.2–0.6)	2.1 (1.8–2.4)
2	4.0	0.4 (0.36–0.39)	0.02 (0.01–0.03)	0.3 (0.33–0.34)	0.7 (0.7–0.8)	4.2 (4.0–4.5)	0.6 (0.6–0.7)	0.02 (0.01–0.04)	0.8 (0.5–1.1)
2	24.0	0.2 (0.22–0.23)	0.05 (0.04–0.06)	0.3 (0.2–0.4)	0.7 (0.6–0.9)	1.5 (1.3–1.7)	0.6 (0.6–0.7)	0.1 (0.07–0.1)	1.1 (0.9–1.3)

group of mice transplanted with fibrosarcoma and injected with $^{64}CuCl_2$ of the same specific activity served as controls.

Groups of three mice bearing the fibrosarcoma were killed by cervical dislocation at postinjection intervals of 15 min, and 1, 4, 24, and 48 hr. Mice bearing the mammary adenocarcinoma were killed at the same time intervals from 15 min through 24 hr. Samples of blood were obtained by heart puncture and placed in preweighed vials. All counting vials contained 2 ml of water. Duplicate tissue samples were excised, weighed, placed in counting vials, and counted in an automatic gamma well counter with a 100-keV window centered on the 511-keV annihilation peak.

An aliquot of the administered dose was counted at the beginning of every experiment, and each sample was corrected for decay to the time of counting the aliquot. The percentage dose/gram was estimated for each tissue.

Rats were transplanted with CSE fibrosarcoma or squamous cell carcinoma of the lung as described. These animals were injected intravenously with 300 μl of Cu-64 KTS solution and killed at the same intervals listed for the mice. Their tissues and blood samples were examined with the same technique.

Radiochemical synthesis. Copper-64 was obtained as a solution in 0.1 *M* HNO_3 with a specific activity of ~1 mCi/mg. This material was evaporated to dryness, redissolved in 0.9% NaCl, and adjusted to pH 2.0 with 0.1 *N* NaOH. KTS dissolved in dimethylsulfoxide was added such that the molar KTS:Cu ratio was 1.2:1, and the mixture was adjusted to pH 7.0 with 0.1 *N* NaOH. The molar concentration of Cu-64 KTS in this solution was typically −1.6 m*M*. Before use, the Cu-64 KTS was diluted with 0.9% NaCl to a specific concentration of about 60 μCi/ml and passed through a 0.22-μ Millipore filter. Radiochemical purity was found to exceed 98% by TLC[¶] using ethylacetate as solvent; R_f for Cu-64 KTS = 0.7, for KTS = 0.65, and for Cu^{2+} = 0.0.

RESULTS

Table 1 shows the data from the mouse experiments.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY FOLLOWING I.v. ADMINISTRATION OF Cu-64 KTS IN FISCHER RATS; (% DOSE/g, MEAN AND RANGE)

No. of animals	Time (hr)	Blood	Fat	Heart	Kidney	Liver	Lung	Muscle	Tumor
				Fibrosarcoma					
3	0.25	0.6 (0.4–0.9)	0.3 (0.2–0.4)	8.6 (8.1–9.3)	5.4 (3.9–7.1)	4.1 (2.9–4.9)	17.6 (11.6–23.4)	0.6 (0.2–1.1)	0.2 (0.1–0.4)
3	1.0	0.8 (0.7–0.9)	0.6 (0.1–1.0)	8.6 (6.5–11.4)	5.1 (4.2–6.2)	7.9 (7.0–10.2)	15.3 (8.6–21.8)	0.4 (0.3–0.4)	0.2 (0.1–0.6)
3	24.0	1.1 (0.8–1.3)	0.3 (0.1–0.4)	3.2 (2.7–3.8)	2.9 (2.2–3.5)	4.4 (2.9–5.9)	1.2 (1.0–1.4)	0.2 (0.1–0.4)	0.4 (0.1–0.8)
4	48.0	1.0 (0.6–1.4)	0.2 (0.1–0.3)	2.8 (2.2–3.7)	2.7 (2.4–3.0)	2.7 (2.4–3.1)	0.9 (0.8–1.1)	0.2 (0.1–0.2)	1.4 (1.0–1.7)
				Lung Squamous Cell Carcinoma					
3	0.25	0.4 (0.3–0.6)	0.04 (0.03–0.06)	5.5 (4.5–8.0)	3.5 (2.0–4.4)	1.8 (1.0–2.3)	14.2 (9.6–21.6)	0.8 (0.2–1.9)	0.4 (0.3–0.5)
3	1.0	0.5 (0.5–0.6)	0.2 (0.1–0.3)	5.6 (5.1–6.4)	7.5 (6.4–8.0)	4.5 (3.6–4.8)	8.2 (6.8–9.9)	0.6 (0.2–0.7)	0.6 (0.5–0.7)
3	4.0	0.6 (0.5–0.6)	0.2 (0.1–0.4)	3.9 (3.2–4.8)	8.5 (7.4–10.0)	6.0 (3.7–8.1)	1.4 (1.1–1.9)	0.3 (0.2–0.4)	0.8 (0.7–0.9)
3	24.0	0.9 (0.5–1.3)	0.2 (0.1–0.4)	3.0 (2.3–3.9)	6.1 (4.7–9.8)	2.7 (2.2–3.3)	0.9 (0.8–0.9)	0.2 (0.2–0.3)	0.7 (0.5–0.9)
4	48.0	1.4 (0.9–2.4)	0.3 (0.1–0.6)	2.6 (2.0–3.1)	4.0 (2.2–4.6)	3.2 (2.7–3.7)	1.0 (0.6–1.2)	0.2 (0.1–0.2)	0.7 (0.3–1.3)

Whereas the mammary adenocarcinoma showed essentially no tendency to concentrate Cu-64 throughout the intervals we studied, an uptake was noted in the fibrosarcoma at 24 and 48 hr. At these times the tumor was the tissue with the highest concentration, reaching 10.1 and 15.4% dose/g, respectively.

At early time intervals, we noted a marked uptake of Cu-64 in the lungs of all the mice. At 15 min, the lung-to-blood ratio ranged from 14–19, and lung-to-liver ratio was 5–9. Low ratios for these tissues at similar times were found in the control animals injected with $^{64}CuCl_2$.

The distribution pattern of $^{64}CuCl_2$ was unlike that for Cu-64 KTS. Most of the $^{64}CuCl_2$ entered the liver within 15 min after injection and remained there. In the control group, Cu-64 did not concentrate in the transplanted tumors or in the lungs.

Table 2 gives the data for the rat experiments. We found no uptake in the squamous cell carcinoma of the lung. The rat CSE fibrosarcoma showed a modest increase in Cu-64 between 24 and 48 hr, reaching 1.4% of the administered dose/g, but this tumor was not the tissue with the greatest concentration at either time interval. The Cu-64 concentrated avidly in the normal lung of the rats at 15 min and 1 hr after injection of the Cu-64 KTS. In the rat, the lung-to-blood and lung-to-liver ratios were greater at 15 min than in the mouse.

DISCUSSION

Copper-64 did not concentrate markedly in either mouse or rat tumor tissue up to 24 hr after injection of Cu-64 KTS, but at 48 hr the mouse fibrosarcoma contained 15.4% of the administered dose/g and the rat fibrosarcoma 1.4% dose/g. It is possible that at 72 hr the rat fibrosarcoma might show a greater concentration, but the 12-hr half-life of Cu-64 precludes its measurement at this late time. A more suitable copper isotope might be Cu-67, with a half-life of 61.7 hr and gamma energies of 92 and 185 keV.

Cu·KTS is a well-characterized metal complex. Its major chemical properties, and probable mechanism of reaction with Ehrlich and sarcoma 180 ascites cells, have been described (*7*). In both cells, the complex reacts with thiols, leaving copper bound within the cells and H_2KTS free to diffuse back into the extracellular medium. This behavior suggested to us that KTS labeled with Cu-64 might prove valuable as a tumor-seeking agent. The uptake of Cu-64 KTS in the mouse fibrosarcoma, at 48 hr after injection, supports this suggestion, although it raises the question of why the other animal tumors did not markedly concentrate the agent.

It would probably be necessary to use a solvent other than dimethylsulphoxide if Cu·KTS is to be administered to humans. In recent work we have found propyleneglycol to be an effective substitute.

FOOTNOTE

* ICN Pharmaceuticals, Inc., Cleveland, OH.

† Radiobiology Laboratory of the Clinical Cancer Center, Madison, WI.

‡ Jackson Memorial Laboratories, Bar Harbor, ME.

‖ Mason Research Institute, Worcester, MA.

§ Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN.

¶ Merck SG60 F254 plates, Merck, Sharp & Dohme.

ACKNOWLEDGMENTS

The authors thank Dr. Kelly Clifton of the Radiation Biology Laboratory, University of Wisconsin, Madison, WI for supplying the MTG-B mammary adenocarcinoma; Dr. Arthur E. Bogden, Mason Research Institute, Worcester, Massachusetts, for the CSE fibrosarcoma; and Dr. Paul Netteshiem, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, for the squamous cell lung tumor used in the rat experiments. We are also indebted to Ms. Jan Johnson for her expert secretarial assistance, and to Dan Kastelic, R.Ph., for help in the preparation of Cu-64 KTS.

This work has been supported in part by Grant No. IN-35 to the University of Wisconsin from the American Cancer Society and Grant No. 5 P0 1-CA 19278 from the National Cancer Institute.

This work was presented in part at the Second International Congress on Nuclear Medicine and Biology, Sept. 17–21, 1978 in Washington, DC.

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