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Autoradiographic Distribution Studies of 2-^{14}C-Fluorouracil following Oral or Intravenous Administration in Mice Bearing Solid Sarcoma-180

JASHOVAM SHANI, JEFFREY A. BERMAN, and WALTER WOLF ˣ

Received September 16, 1976, from the *Radiopharmacy Program, School of Pharmacy and Cancer Research Center, University of Southern California, Los Angeles, CA 90033.* Accepted for publication June 21, 1977.

Abstract □ 2-^{14}C-Fluorouracil was injected or fed to mice bearing sarcoma-180, a tumor sensitive to fluorouracil, and the fate and localization of the label were followed for 2 hr. The disappearance of the label from the blood and its localization in the tumor and organs of these mice were studied by autoradiography as a model for scintigraphic localization. In tumor-bearing mice, the tumor, kidney, liver, and bladder were visualized 10 min after intravenous injection of 2-^{14}C-fluorouracil; 2 hr after the injection, the activity in the tumor and bone marrow was still noticeable. The localization in the tumor and the liver appeared visually to be rapid, and the retention of the label in the liver of the tumor-bearing animals was noticeably greater than in the control mice by visual observation. There seemed to be no difference in the distribution of 2-^{14}C-fluorouracil when the drug was administered orally to starved or to fed mice. The tumor was visualized equally after oral administration as well as after intravenous injection, except for a somewhat faster clearance from most organs in the intravenous group. There was no difference in uptake and excretion if additional carrier was added, which doubled the administered dose of fluorouracil per mouse. Inasmuch as most of the activity viewed in the sarcoma-180 bearing mice is known to be due to the metabolite floxuridine monophosphate, a correlation between the tissue localization of the drug and its clinical efficacy may lead to a method for predicting the chemotherapeutic regimen in patients. The present work attempted to determine animal data relevant to a nuclear medicine observation.

Keyphrases □ 2-^{14}C-Fluorouracil—tissue distribution in mice after oral or intravenous administration, autoradiographic determination □ Autoradiography—determination, 2-^{14}C-fluorouracil, tissue distribution in mice after oral or intravenous administration □ Distribution, tissue—2-^{14}C-fluorouracil in mice after oral or intravenous administration, autoradiographic determination □ Antineoplastic agents—2-^{14}C-fluorouracil, tissue distribution in mice after oral or intravenous administration, autoradiographic determination

Fluorouracil[1] has been indicated since 1958 for treatment of patients with primary carcinoma of the breast, stomach, or colon with metastases to the liver (1). However, there are different views on its optimal chemotherapeutic regimens, *i.e.*, acute loading *versus* weekly therapy, rapid *versus* slow infusion, oral *versus* intra-arterial administration, and regional infusion *versus* radiological adjunct therapy (2).

BACKGROUND

Due to the inconsistency and variability of the clinical response to fluorouracil, the regimen of its systemic administration is controversial. For the past 12 years, the following regimens have been tested. Oral daily administration proved effective in patients with liver metastases due to the high concentration in the portal system (3–5). In cases of rectal carcinoma, oral administration was replaced by rectal instillation (6). Intravenous weekly administration (without a loading dose) maintained antitumor effectiveness and reduced toxicity in studies with 437 patients (7–9) and in a cumulative study of 548 patients with disseminated cancer (10). Intra-arterial infusion, through a catheter into the external carotid artery, the hepatic artery, or the bronchial artery, exhibited improvement in over 50% of patients (11, 12). Intralymphatic injection was tested for lymphoreticulosarcoma and lymph node metastases (13). Intralumenal infusion into sequestered intestinal lumen prior to surgery (14) and intramuscular and intraperitoneal (15) injections also were evaluated.

In the first comprehensive study of a regimen of choice in cancer patients, Mukherjee *et al.* (15) administered 2-^{14}C-fluorouracil by oral, intravenous, intramuscular, and intraperitoneal routes. They measured the radioactivity in the plasma, respiratory carbon dioxide, and urine and observed that the unchanged drug was detected in the urine following intravenous administration longer than after administration by any other route.

Since the intravenous and oral routes remained the preferred methods for administering fluorouracil, most recent studies compared those two routes. Bateman *et al.* (16) found a clinically useful response rate in 21% of the intravenous group and in 40% of the oral group. Although the response duration for both groups was not significantly different, great variability in peak plasma level and decay was observed after oral administration, while the range of peak levels and plasma decay in the intravenously administered patients was relatively uniform (17). Cohen *et al.* (18) reported that patients receiving intravenous doses showed consistent and rapid plasma clearance, while patients demonstrated widely varying peak plasma concentrations after oral ingestion.

Comparison of therapy regimens after intraperitoneal administration of fluorouracil to mice bearing L-1210 solid lymphocytic leukemia demonstrated essentially no schedule dependency; this finding is consistent with the effectiveness of weekly doses (19). It was suggested that the optimal treatment might be injections of fluorouracil every 2 days

[1] 5-Fluorouracil.

after tumor inoculation (19). These data suggest single weekly doses of fluorouracil rather than intensive monthly courses.

These variations, plus the fact that fluorouracil remains the drug of choice for a significant number of neoplasms, led to a study of the distribution of ^{18}F-fluorouracil to optimize and individualize chemotherapeutic regimens.

Naturally, no disposition or organ distribution after intravenous or oral administration can be studied in a large number of humans. Therefore, the distribution of 2-^{14}C-fluorouracil as a function of these two administration routes was studied in a model animal bearing a tumor responsive to the drug: a solid sarcoma-180. The goals of this research were to: (*a*) evaluate the time course of distribution of fluorouracil in fluorouracil-responsive tumor-bearing mice, (*b*) evaluate the intravenous *versus* oral route of administration, (*c*) determine whether there is any difference if fluorouracil is given before or after meals, (*d*) decide whether or not a loading dose is necessary for fluorouracil absorption and distribution in the two groups tested, and (*e*) study drug distribution in the organs.

An autoradiographic method was chosen so that ^{14}C-visualization could be related to subsequent ^{18}F-localization by scintigraphic methods. While this technique is of interest for total body visualization of organs, it is also the closest analogy to the manner in which the nuclear medicine scan "sees" the body and its organs. Indeed, the same arguments concerning quantitation (or lack of it) can be leveled against both procedures; but to determine if a given agent can be "visualized" by a scan, it is useful to determine if it can be visualized by an autoradiographic tomographic cut. In addition, the present development of positron emission tomographic procedures make autoradiography the logical model of such animal model studies[2].

EXPERIMENTAL

Sixty male Swiss-Webster mice[3], 18–22 g, were implanted subcutaneously with sarcoma-180 cell suspension. Seven days later, they were divided into three equal groups: a, b, and c. Sixty additional control male mice were divided into three additional groups: a, b, and c. All 120 mice received 2-^{14}C-fluorouracil[4], 10 μCi/0.15 mg. Group a received the drug intravenously; Group b was given the drug orally while on a regular feeding diet; Group c was given the drug orally after 18 hr of fasting.

The mice in each group were divided into five equal subgroups and were rapidly sacrificed and frozen in dry ice–hexane at 10, 15, 30, 70, or 120 min after the drug was administered. Twenty-four additional mice (12 transplanted and 12 controls) received, in addition to the same regimen, 0.15 mg of unlabeled fluorouracil as carrier and were sacrificed and frozen 2 hr after injection. These procedures follow a standard whole-body autoradiographic methodology (21).

The mice were placed overnight in a freezer (−20°). The legs and tail were dissected, and each mouse was mounted sagitally on an aluminum block and covered with carboxymethylcellulose. These procedures must be carried out below 0°. The embedded mice were then placed in the freezer for 10–30 hr to allow the carboxymethylcellulose to harden. The animals were cut into 30-μm sections, and every 10th section was placed on a cellulose tape. The sections were freeze dried on glass plates; they were then exposed to a photographic film[5] for 10–14 days, developed in an X-omat system, and analyzed.

For a semiquantitative analysis of the whole-body autoradiograms, a 0–6 scale was established. The higher the figure, the higher is the electron density of the organ evaluated on the film. This scale is based on standard radiological procedures. Each film was evaluated by two experienced technicians; to minimize intraexperimental variations, all films were read *en bloc* at the end of the experiment. The liver, stomach, intestine, kidney (cortex and medulla), bladder, bone, tumor (periphery and center), and blood were evaluated. All animals were manipulated under the same experimental conditions, and all evaluations were given absolute readings.

RESULTS

Intravenous Administration—The first organ that visually showed concentrations of the radioactivity of 2-^{14}C-fluorouracil in the control mice was the kidney, where the activity appeared to be significant 10 min postinjection, followed 5 min later by observable concentrations in the bladder and liver. Forty-five minutes postinjection, significant radioactivity was visualized in the liver, the bladder showed a heavy concentration, and significant kidney uptake was seen, especially in the renal medulla.

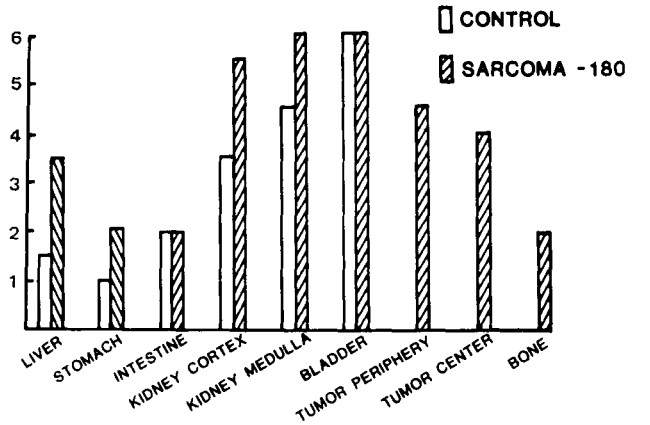

Figure 1—*Distribution of radioactivity following administration of 10 μCi of 2-^{14}C-fluorouracil. Both the control mice and the mice bearing sarcoma-180 were sacrificed 30 min after the intravenous administration of the radioactive drug.*

In the tumor-bearing group, the tumor, kidney, liver, and bladder were visualized as early as 10 min after injection. The observable concentration in the tumor remained steady, but the activity in the kidney decreased with time. Two hours after injection, there was only slight liver and kidney visualization, but the activity in the tumor and bone marrow was still significant. In the tumor-bearing animals, there apparently was an early (10-min) liver and tumor concentration of radioactivity, and the retention in the liver was noticeably greater than in control mice. The bladder activity in the tumor-bearing mice was not substantial until 30 min after injection. Representative readings for the intravenous results are shown in Figs. 1 and 2.

Oral Administration—There was considerable localization in the stomach, intestine, kidney, and bladder 15 min after oral administration. There was no significant visual difference in uptake between the fed and starved animals, except for an earlier clearance from the empty stomachs of the starved mice. The tumor was visualized 10 min after drug administration at the same rate and intensity as following intravenous administration, with a peak 30 min postinjection. The tumor was still visualized 2 hr after oral drug administration; however, its level of localization was significantly less than in the intravenously injected animals.

There was essentially no significant difference in the uptake of the orally administered drug between the fed and starved groups or, at 2 hr postinjection, between the groups with or without additional fluorouracil carrier. Activity in the nasal mucosa was observed in most animals, corresponding to similar findings in humans. Representative values for the

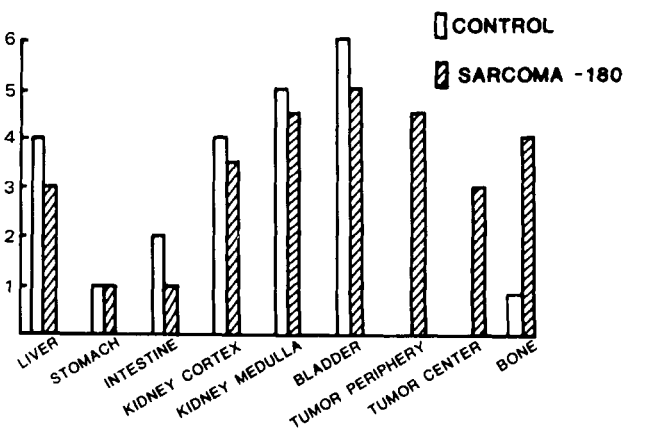

Figure 2—*Distribution of radioactivity following administration of 10 μCi of 2-^{14}C-fluorouracil. Both the control mice and the mice bearing sarcoma-180 were sacrificed 70 min after the intravenous administration of the radioactive drug.*

[2] Preliminary results of this study were presented at the 1975 meeting of the Society of Nuclear Medicine (20).
[3] Jackson Laboratories, Bar Harbor, Me.
[4] Biochemical and Nuclear Corp., Burbank, Calif.
[5] Kodak RP-54.

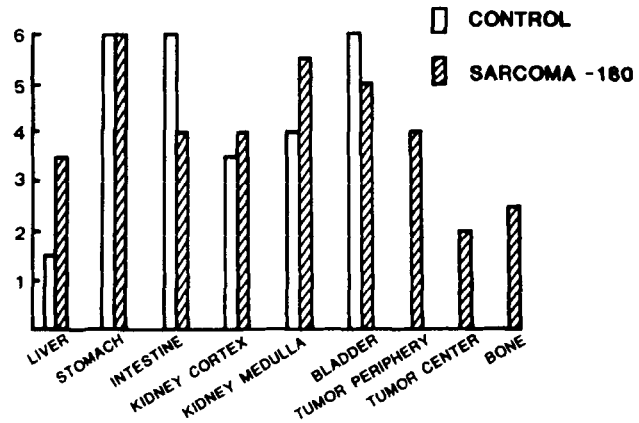

Figure 3—*Distribution of radioactivity following administration of 10 μCi of 2-^{14}C-fluorouracil. Both the control mice and the mice bearing sarcoma-180 were fed* ad libitum *and sacrificed 30 min after oral administration of the radioactive drug.*

fed mice are given in Figs. 3 and 4; the retention capacity of the label, with time, in the tumor periphery of all three groups is shown in Fig. 5. Figure 6 shows the variation in the tumor periphery/center as a function of time.

DISCUSSION

The solid tumor sarcoma-180 in Swiss mice was chosen due to consistent reports about its responsiveness to fluorouracil. This drug was reported to cause significant inhibition of the tumor (22) as well as morphological changes (23). In one study, the sarcoma-180 tumor had the highest observable retention of 2-^{14}C-fluorouracil of all tissues examined (24), and it showed high conversion of fluorouracil into nucleotides (25). It suited the present study because there was maximal observable tumor localization 70 min after intravenous injection, which decreased slowly during the 2nd hr, and this decrease was within a reasonable experimental time period.

The 0.5–2-hr plateau in tumor activity had been noticed before (26), but there is a difference in visual binding dynamics between the center and the periphery. The persistence of fluorouracil in this tumor appears to be consistent with the clinical effectiveness of the drug against some slow-growing human tumors, especially due to the fact that much of its activity was concentrated in the cortex, rather than in the medulla of the tumor, as could be expected due to the necrotic nature of the tumor (Fig. 6). Higher ratios between outer and inner areas of the tumor were reported in prolonged (3-day) experiments (27). By 72 hr, floxuridine (2′-deoxy-5-fluorouridine) monophosphate was the metabolite present in most tissues in the highest concentrations (27).

The bone marrow revealed its peak activity in the present experiments 2 hr after injection. In the animals that were mounted obliquely so that the spinal column was noticeable, marrow localization appeared to be

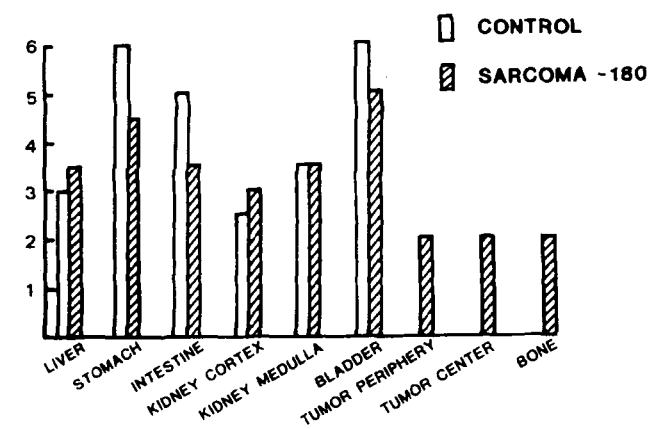

Figure 4—*Distribution of radioactivity following administration of 10 μCi of 2-^{14}C-fluorouracil. Both the control mice and the mice bearing sarcoma-180 were fed* ad libitum *and sacrificed 70 min after oral administration of the radioactive drug.*

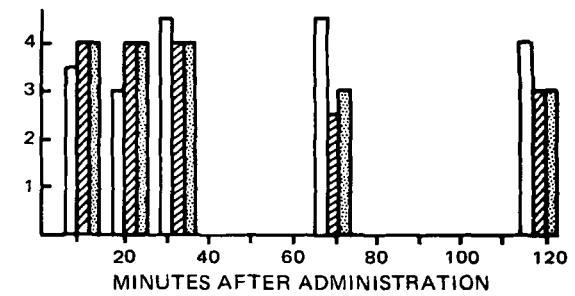

Figure 5—*Retention of radioactivity from 2-^{14}C-fluorouracil in the tumor periphery of mice bearing sarcoma-180 at various intervals after oral or intravenous administration of 10 μCi of the radioactive drug. Key:* □, *intravenous;* ▨, *oral to fed; and* ■, *oral to starved.*

more intense in tumor-bearing animals after intravenous administration. This result correlates to marrow uptake after fluorouracil administration in humans.

The brain did not concentrate any activity during the experimental period. Prolonged studies of 24 hr and more demonstrated specific localization in the granulosa layer of the cerebellum (27). A similar situation was seen in the pancreas, which concentrates the drug with time, but no pancreatic activity could be noticed in the present studies.

Blood clearance was rapid and in agreement with a one-compartment kinetic model in humans (18). In humans, plasma clearance after oral administration is much slower than after intravenous administration; the plasma peak is in approximately 1 hr as compared to the intravenous $T_{1/2}$ of 10 min (18). Although the differences in the plasma time course of free fluorouracil after oral or intravenous administration do not reflect the difference in clinical response associated with those routes (16), it might be a reliable parameter for the availability and metabolism in each patient.

The liver is apparently the main site of fluorouracil degradation. In humans, there is more extensive catabolism when the drug is administered orally rather than intravenously (25). There was a significant delay in liver uptake in tumor-bearing mice as well as a rapid clearance in the control mice, probably due to impaired metabolism in the tumor-bearing animals. It is suggested that the delayed concentration in the bladder of the tumor-bearing animals is another result of this high liver uptake and slow metabolism. The concentration in the kidney is an outcome of this relationship, and fluorouracil is concentrated in the kidney's medulla.

CONCLUSIONS

It is apparent that intravenous injections of fluorouracil localize more specifically in the tumor and bone marrow sites, as noted by visual observation, and that the apparent drug retention at these sites is greater than after oral administration. The fact that the bladder activity in an injected animal occurs at a later time than in the orally administered animal, and considering the interrelationship between toxicity and degradation, it seems that fluorouracil toxicity is diminished by an increase in its degradation. There was less degradation after the continuous intravenous infusion of 5-fluorodesoxyribose than after a single intravenous dose, and 5-fluorodesoxyribose toxicity was increased greatly when injected as a continuous intravenous drip (28). A study to determine the optimal chemotherapeutic dose and regimen of fluorouracil administration, using γ-emitting 5-^{18}F-fluorouracil, is now being conducted;

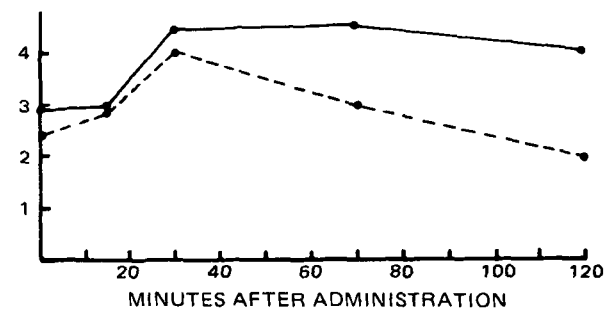

Figure 6—*Distribution of radioactivity from 2-^{14}C-fluorouracil in the sarcoma-180 tumor tissue of mice during the first 2 hr following intravenous administration of 10 μCi of the radioactive drug. Key:* ●—●, *tumor periphery; and* ●- -●, *tumor center.*

results comparing the localization of a responsive *versus* a nonresponsive tumor were published elsewhere (29).

The present study also models the observations made by a nuclear medicine scan, where visual differences of film contrast (scales of gray) are the bases for clinical diagnosis. Thus, because visual differences are observable, it is expected that similar visual differences may be observable in the human. In addition, the use of ^{18}F-fluorouracil-labeled material in conjunction with tomographic cuts obtained with the new positron emission tomographic scanners is likely to produce scans whose interpretation can be correlated closely to the present work.

Although the question of whether localization and utilization of chemotherapeutic agents are an indication of their pharmacological activity is a matter of controversy, studies correlating these factors will help in predicting a more appropriate fluorouracil chemotherapeutic regimen.

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Isolation and Characterization of the Sesquiterpene Lactones Costunolide, Parthenolide, Costunolide Diepoxide, Santamarine, and Reynosin from *Magnolia grandiflora* L.

FAROUK S. EL-FERALY ˣ and YEE-MING CHAN

Received February 7, 1977, from the *Pharmacognosy Department, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication June 27, 1977.

Abstract □ The germacranolide sesquiterpene lactones costunolide, parthenolide, and costunolide diepoxide were isolated from the leaves of *Magnolia grandiflora* L. Costunolide diepoxide might be, at least in part, an artifact derived from air oxidation of parthenolide. The root bark yielded only costunolide together with the two eudesmanolides, santamarine and reynosin. In an attempt to synthesize costunolide diepoxide, the action of *m*-chloroperbenzoic acid on parthenolide and on costunolide was studied. The products were costunolide diepoxide from parthenolide and the two cyclized derivatives, santamarine and reynosin, from costunolide. The elusive costunolide 1,10-epoxide was obtained by epoxidizing costunolide using a biphasic system containing sodium bicarbonate. Under these conditions, epoxidation of costunolide took place without cyclization.

Keyphrases □ *Magnolia grandiflora* L.—leaves, isolation and characterization of various sesquiterpene lactones □ Lactones, various sesquiterpene—isolated and characterized from leaves of *Magnolia grandiflora* L.

Numerous cytotoxic sesquiterpene lactones have been isolated from plants belonging to the Magnoliaceae family (1–4). *Magnolia grandiflora* L., a member of this family, is commonly known as Southern Magnolia (5). This large evergreen ornamental tree is widespread throughout the United States (5).

Only the sesquiterpene parthenolide (I) was reported to occur in this plant (4). The present studies revealed the presence of additional sesquiterpenes in the leaves and root bark. These studies were carried out as a part of a random screening program of local flora for biologically active compounds.