A Model for Prediction of Chemotherapy Response to 5-Fluorouracil Based on the Differential Distribution of 5-[^18F]Fluorouracil in Sensitive versus Resistant Lymphocytic Leukemia in Mice

Jashovam Shani and Walter Wolf

Radiopharmacy Program and Cancer Research Center, University of Southern California, Los Angeles, California 90033

SUMMARY

The distribution of 5-[^18F]fluorouracil has been compared in two variants of the same tumor in C57BL x DBA/2 F, mice: solid L1210 lymphocytic leukemia tumor susceptible to 5-fluorouracil treatment and the same tumor, made resistant to the drug over a 34-generation span. Significant differences in 5-[^18F]fluorouracil distribution were observed, most notably in the tumor-blood ratios at 12 hr postinjection. The drug-responsive tumor showed a 20:1 concentration ratio, whereas the drug-resistant tumor only had a 4:1 concentration ratio. We postulate that these differences, observed here in this animal tumor model, may be a reflection of similar ratio differences in humans. This technique may allow, by noninvasive quantification of tumor-blood ratios following administration of 5-[^18F]fluorouracil to man, the differentiation of those human tumors that are likely to respond to drug therapy from those in which the response will be minimal or nil.

INTRODUCTION

Although 5-FU is considered a drug of choice in treating certain types of disseminated human cancer, its efficacy in those patients is about 30%, i.e., 7 of every 10 cancer patients treated with this drug either show no favorable effect to 5-FU or terminate before any regression can be recorded. We had suggested (7) a new technique, which we have denominated “radiopharmacokinetics,” that may be of use in the prediction of drug effectiveness and determination of the dosage and regimen required with various potent antitumor agents. This technique, which aims to make available the tools of nuclear medicine, namely, external detection of gamma-emitting radionuclides using noninvasive techniques, to the service of the clinical chemotherapist, is based on the use of short-lived gamma-labeled radiopharmaceuticals that are radioisomers or mimetic analogs of the drug whose distribution we attempt to study.

The specific example that we wish to report here deals with the differences observed in the distribution of a gamma-labeled isomer of 5-FU, 5-[^18F]FU, between 2 lines of solid L1210 lymphocytic leukemia: L1210/0 (susceptible to 5-FU treatment) or L1210/5-FU (resistant to the drug).

MATERIALS AND METHODS

Tumor Models. The L1210/0 variant was obtained from Arthur D. Little, Cambridge, Mass. The leukemia L1210/5-FU variant (solid) used in these studies was developed at the Animal Tumor Research Facility of the LAC/USC Cancer Center by T. Khwaja and J. Varven, from L1210-X111/5-FU ascites (developed by Dr. Dorris Hutchison at the Sloan-Kettering Institute, N. Y.) obtained from Arthur D. Little, Cambridge, Mass. The technique involves daily treatment of DBA/2 mice bearing solid L1210/0 with 40 mg of 5-FU per kg for 30 transplant generations and then with 5 daily injections per week of 10 mg of 5-FU per kg for 4 additional transfer generations. After 34 transplant generations the L1210/5-FU mice displayed complete resistance to 5-FU (T. Khwaja, personal communication), and their life span after inoculation was 8.5 days, the same as for the nontreated sensitive line. Nevertheless, they were retested for lack of antitumor effect by 5-FU injected daily at 10 mg/kg. No variation in life-span was observed (T. Khwaja, personal communication). The stock tumor lines for both variants were maintained in DBA/2 mice, and the tumors were implanted in C57BL x DBA/2 F1 (hereafter called BD2F1) mice for the experimental studies. Female BD2F1 mice, weighing 20 g each, were implanted s.c. with approximately 40-mg fragments of solid L1210 lymphocytic leukemia, taken from the DBA/2 mice on the 7th day after inoculation (the day of inoculation designated as Day 0). The fragments were implanted in the axillary region of the flank, as described by Chadwick and Rogers (2). On the 6th day of tumor growth the mice were given a single i.v. injection of 5-[^18F]FU via the tail vein, and the animals were bled and sacrificed 2 or 12 hr postinjection.

Radiopharmaceutical Preparation. 5-[^18F]FU was synthesized in our laboratory according to a method developed by

1 Supported by National Cancer Institute Grants PO1-CA-14089 and PO1-CA-19438.
2 International Atomic Energy Agency Fellow. Present address: Radiopharmacy Laboratory, School of Pharmacy, The Hebrew University, POB 12065, Jerusalem, Israel.
3 To whom requests for reprints should be addressed.
* The abbreviation used: 5-FU, 5-fluorouracil.
Received August 23, 1976; accepted April 6, 1977.
Chemotherapy Prediction for 5-FU

Chemotherapy Prediction for 5-FU

Fowler et al. (3), as detailed in a recent publication by Shani et al. (6). The specific activity of the preparations was maintained constant for the different batches at 1 µCi/µg.

Animal Distribution Studies. Each mouse that was to be sacrificed after 2 hr received 10 µCi 5-[^18F]FU i.v. to the tail vein, and those to be sacrificed 12 hr postinjection received 400 µCi i.v. These differences were necessary in order to uniformize counting statistics. Although this resulted in a 40-fold difference (10 to 400 µCi/mouse) of carrier 5-FU used, previous collaborative experiments that were carried out simultaneously in our laboratory and at the Brookhaven National Laboratory (6) had suggested that the amount of 5-FU carrier in that range (10 to 1000 µCi/mouse) did not appear to affect the distribution of 5-[^18F]FU in the L1210 tumor-bearing mice or in the other tumor-bearing mice tested (5, 6).

The mice were bled by a heart puncture while under light diethyl ether anesthesia; subsequent to cervical dislocation, the tumor, as well as the lung, liver, spleen, pancreas, adrenals, kidneys, uterus, thyroid, and heart were excised and weighed. Aliquot portions of the femur and the leg muscle were also sampled and weighed. All samples, as well as 2 radioactive 5-[^18F]FU standards per mouse, were counted for 1 min each in a Beckman Biogamma counter, and the results obtained are expressed as percentage injected per g, from which the organ: blood ratios and percentage injected per organ were calculated. No decay calculations were needed, since both samples and standards were counted simultaneously. Six sets of experiments were performed; both the resistant and the responsive lines were tested concurrently so that we could evaluate both the differences between the 2 variants in each single experiment and those between the 2 accumulated groups, and to demonstrate in this way a high interassay reproducibility.

RESULTS AND DISCUSSION

The results of this study are summarized in Table 1. The difference in retention of the radioactive label in the tumor between the 2 tumor lines at 2 hr postinjection has a factor of 2, whereas the same factor at 12 hr postinjection is 5.1 (p < 0.001), thus indicating that, not only the "sensitive" tumor line retains significantly more activity than does the resistant line, but the difference in the 2 lines is a function of time. The mean tumor: blood ratio in the L1210/0 (5-FU sensitive mice) of 20.60 at 12 hr postinjection is the highest tumor: blood ratio so far obtained in animal tumor studies with 5-[^18F]FU and suggests that if these distribution ratios in mice are somewhat similar in man, differential visualization of human tumors comparing those sensitive and resistant to 5-FU chemotherapy may be feasible.

Not only were the differential ratios between the sensitive and resistant variants significantly higher at 12 hr postinjection than at 2 hr postinjection, but, for the sensitive tumor at the later period (12 hr) no other soft tissue had an organ: blood ratio higher than the tumor, whereas at the earlier period (2 hr) both major metabolic organs, the liver and

---

Table 1

5-[^18F]FU distribution in female BDF1 mice bearing L1210 lymphocytic leukemia 2 or 12 hr after i.v. injection

<table>
<thead>
<tr>
<th>Organ</th>
<th>L1210/0 (2 hr)</th>
<th>L1210/5-FU (2 hr)</th>
<th>L1210/0 (12 hr)</th>
<th>L1210/5-FU (12 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.60 ± 0.10</td>
<td>0.92 ± 0.19</td>
<td>0.60 ± 0.10</td>
<td>0.92 ± 0.19</td>
</tr>
<tr>
<td>Thyroid</td>
<td>7.65 ± 0.20</td>
<td>11.23 ± 0.29</td>
<td>7.65 ± 0.20</td>
<td>11.23 ± 0.29</td>
</tr>
<tr>
<td>Liver</td>
<td>4.32 ± 0.09</td>
<td>1.03 ± 0.03</td>
<td>4.32 ± 0.09</td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.40 ± 0.04</td>
<td>1.40 ± 0.04</td>
<td>1.40 ± 0.04</td>
<td>1.40 ± 0.04</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.78 ± 0.09</td>
<td>0.78 ± 0.09</td>
<td>0.78 ± 0.09</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.66 ± 0.09</td>
<td>0.66 ± 0.09</td>
<td>0.66 ± 0.09</td>
<td>0.66 ± 0.09</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.70 ± 0.03</td>
<td>0.70 ± 0.03</td>
<td>0.70 ± 0.03</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>Bone</td>
<td>3.86 ± 0.22</td>
<td>1.90 ± 0.12</td>
<td>3.86 ± 0.22</td>
<td>1.90 ± 0.12</td>
</tr>
</tbody>
</table>

* The availability of the Energy Research and Development Administration cyclotron at UCLA for the production of 18F is deeply appreciated.
J. Shani and W. Wolf

kidneys, had organ:blood ratios higher than those of the tumor. This finding suggests that such longer times are to be used for assessing any differences in tumor-concentrating ability. Although these 12-hr determinations in the present models of tumor-bearing mice provided significant and quantitatable values of both high tumor: blood ratio and good clearance from the other soft tissues, more work is necessary to determine the optimal time of observation, when tumor:blood ratio is highest, while the decay of $^{18}$F ($t_{1/2}, 2$ hr) still provides adequate statistics to differentiate tissues. It is likely that such timing may be optimal anywhere between 6 and 16 hr, suggesting that this technique could be used in man for evaluating: (a) whether the tumor:blood ratios measured in a given patient are consistent with his response to 5-FU therapy or, for that matter, to any other gamma-labeled chemotherapeutic agent; (b) what is likely to be the optimal dosage that, while providing the highest tumor concentration, will also elicit the maximum amount of side effects; and (c) the duration of the possible response, so as to determine the rate of drug administration.

Very few time-study measurements of labeled 5-FU distribution in human tumors had been reported in the literature; therefore it is interesting that our results agree well with a preliminary report (1) on 5-[2-$^{14}$C]FU administration to a single cancer patient (male, 43 years old, with anaplastic lung carcinoma), where “the maximum specific activity of the tumor was at 12 hours . . . parallel to patterns observed in mice.” Some efficacy studies of antileukemic agents in mice bearing L1210/0 and L1210/5-FU tumors are summarized by Kline et al. (4).

On the basis of this study, it is suggested that further studies be conducted to determine whether 5-$[^{18}$F]FU can be used as a diagnostic agent for experimental prediction of responsiveness to 5-FU chemotherapy in humans. We project that a dose of 5 to 10 mCi will be required for a human study, if the optimal time of observation falls in the 6- to 10-hr range, and 15 to 25 mCi if it extends beyond 12 hr. These doses are within well acceptable ranges for radiation dosimetry.

REFERENCES

A Model for Prediction of Chemotherapy Response to 5-Fluorouracil Based on the Differential Distribution of 5-[\textsuperscript{18}F]Fluorouracil in Sensitive versus Resistant Lymphocytic Leukemia in Mice

Jashovam Shani and Walter Wolf


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/Part_1/2306

---

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/37/Part_1/2306. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.