Response of DNA Thymine Synthesis in Human Tumor and Normal Tissue to 5-Fluorouracil

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SUMMARY

Human tumor tissue and the normal tissue from which it arose similarly incorporated DNA thymine precursors. The effect of 5-fluorouracil on alternative pathways of DNA thymine synthesis was similar in both tumor and normal tissue. Differential drug sensitivity was not demonstrated.

INTRODUCTION

This study investigates the premise that human solid tumors divide more rapidly than do their normal tissue counterparts and are therefore more sensitive to drugs that inhibit DNA synthesis. The amount of drug that can be administered in tumor chemotherapy is limited by the toxicity resulting from normal tissue destruction (1, 8, 11). For chemotherapy to be effective, it would seem that the tumor should be more drug sensitive than the normal tissue from which it arose. Such differential drug sensitivity has not been demonstrated and is the subject of the present study.

The effect of 5-fluorouracil in biological systems is thought to be due to its inhibition of thymidylate synthetase (4, 5, 10). Thymidylate synthetase is an important enzyme in the major synthetic pathway for DNA thymine. It catalyzes the addition of single-carbon units at the 5 position of dUMP to form TMP (2, 3). Formate and carbon 3 from serine are sources of such single-carbon units. Incorporation of 1-carbon units into DNA thymine decreases when thymidylate synthetase is inhibited. This provides a means for assessing the biochemical effect of 5-fluorouracil. However, thymidylate synthetase is required for only 1 of the 2 alternative pathways for DNA thymine synthesis. In the other pathway, thymidine is phosphorylated to TMP by thymidine kinase, and thymidylate synthetase is not required. Cellular regulatory mechanisms control thymidine kinase activity. A decrease in the intracellular level of TMP results in increased thymidine kinase activity (6, 9). Intracellular TMP decreases when thymidylate synthetase is inhibited by 5-fluorouracil. In response, thymidine kinase activity and thymidine incorporation increase. Therefore, accurate assessment of the effect of 5-fluorouracil on DNA thymine synthesis requires simultaneous evaluation of both alternative pathways for TMP synthesis.

This study investigated the alternative pathways in vitro in human tumors and in autochthonous normal tissue.

MATERIALS AND METHODS

The methods used in this study have been previously described (12). In summary, tissues obtained from patients who had not received prior chemotherapy were minced promptly after surgical removal. In the case of normal bowel tissue, only the epithelium was used for the studies. Duplicate incubations containing either no drug or 5-fluorouracil, 1.8 X 10^{-7} or 1.8 X 10^{-6} M, were prepared. After 1 hr, thymidine-methyl-3H (19.5 Ci/m mole) and either sodium formate-14C (25 mCi/m mole) or serine-3-14C (48 mCi/m mole) were added. Incubation flasks were removed after 23 hr. The medium was removed by centrifugation, and 4% perchloric acid was added. Acid-soluble compounds were removed, and the DNA was isolated. Hydrolysis in formic acid liberated the free bases from DNA. Paper chromatography with carrier added was used to isolate the thymine. The amount of each labeled precursor present in the thymine spot was determined by dual-label liquid scintillation counting. Conversions to n moles incorporated per ml of tissue were calculated on the basis of precursor specific activity and the volume of tissue used in each incubation.

RESULTS

It was necessary to establish that the biological activity of 5-fluorouracil was accurately reflected in the observed effects on precursor incorporation into DNA thymine. The effect of 5-fluorouracil on incorporation of 1-carbon units from 2 different sources was compared in a number of tumors (Chart 1). At the same 5-fluorouracil concentrations, formate-14C and serine-3-14C incorporations were similarly inhibited. The compensatory increase in thymidine incorporation observed in the presence of 5-fluorouracil was further evidence that the data obtained accurately reflected drug effect (Charts 2 and 3). In view of these findings, serine-3-14C and thymidine-methyl-3H were used in subsequent experiments as DNA thymine precursors.

The uptake patterns and drug effect were strikingly similar in colon tumors and autochthonous normal tissue from 7 patients.
Chart 1. Comparison of various 5-fluorouracil concentrations on formate-14C and serine-3-14C uptake into DNA thymine in 28 human solid tumors. Serine values on the abscissa and formate values on the ordinate are expressed as (log10 dpm without 5-fluorouracil)-(log10 dpm with 5-fluorouracil). The various drug concentrations (M) are: 0, 1.8 x 10^-8; *1.8 x 10^-7; a, 1.8 x 10^-6; *, 1.8 x 10^-5.

Chart 2. Effect of 5-fluorouracil on serine-3-14C (•) and thymidine-methyl-3H (□) incorporation into DNA thymine in human normal and colon tumor tissue. Uptakes in each tissue are depicted as a series of 3 double bars. The double bars in each group represent, from left to right, uptake at 5-fluorouracil concentrations of 0, 1.8 x 10^-8, and 1.8 x 10^-4 M. The groups of bars represent, from left to right, labeled precursor uptake in normal colon tissue from 1 patient without colon carcinoma (Patient 1) and 7 patients with colon carcinoma (Patients 2 to 8). Autochthonous tumor uptakes are located above their normal tissue counterparts. Uptakes are expressed as nmoles of DNA thymine synthesized from the labeled precursors in 24 h/3 ml of tissue.

Chart 3. Effect of 5-fluorouracil on serine-3-14C (•) and thymidine-methyl-3H (□) incorporation into DNA thymine in human normal tissues and various tumor tissues. Data are presented as in Chart 2, except that from left to right are depicted uptakes in 3 breast carcinomas (1 to 3), 1 lung carcinoma (4), and 1 ovarian carcinoma (5).

Chart 4. Effect of delay in initiating incubation on serine-3-14C (•) and thymidine-methyl-3H (□) uptake in 4 normal and 4 autochthonous tumor tissues. The period of delay is given beneath each double bar.
patients (Chart 2). In the single specimen obtained from a person without carcinoma, the uptake pattern was very similar to that found in patients with carcinoma. Tumor and autochthonous normal tissue uptake were similar in 5 other carcinomas: 3 of the breast, 1 of the lung, and 1 of the ovary (Chart 3).

Uptake values varied by a factor of 10 in the bowel specimens studied. Possibly, this amount of variation could have been due to differences in the amount of time that circulation was compromised in removal of the tumor. For investigation of this possibility, 4 tumors were rapidly resected and allowed to remain at room temperature for various intervals. Uptakes in tumors which stood for intervals of 1 or 2 hr were found slightly decreased compared with those obtained immediately after removal (Chart 4). This amount of the decrease, however, was insufficient to explain the differences noted in basal uptakes in the various tumors.

DISCUSSION

The activity of 5-fluorouracil is determined by the cellular concentration of the active metabolite of the drug, 5-fluoro-2'-dUMP, and the efficiency with which that drug concentration blocks thymidylate synthetase. The balance between anabolic activation of 5-fluorouracil and its catabolic degradation determines the cellular level of 5-fluoro-2'-dUMP. In 2 of 3 patients given i.v. 5-fluorouracil-2,14C and who underwent surgery for colon cancer, Mukherjee et al. (7) found higher levels of radioactivity in tumor than in autochthonous normal tissue. In the other patient given 5-fluorouracil and in 2 additional patients given 5-fluoro-2'-deoxyuridine-2,14C, radioactivity was the same in both colon tumor and normal tissue. Nucleotide levels were greater in the tumor in 1 of the patients given 5-fluorouracil-2,14C and in both patients given 5-fluoro-2'-deoxyuridine-2,14C. The present study indicates that 5-fluorouracil anabolism, catabolism, and inhibition of thymidylate synthetase are similar in both types of tissues.

Tumor tissue appeared to carry with it characteristics of DNA synthesis of the organ from which it arose. In vitro sensitivity to 5-fluorouracil was almost identical in tumor and in normal tissue. Synthesis of DNA thymine and sensitivity of this synthesis to 5-fluorouracil were similar in colon tissues from persons with and without cancer. Human solid tumors neither synthesized DNA thymine more rapidly nor were more sensitive to 5-fluorouracil than were corresponding normal tissues. It may be concluded either that differences in biochemical sensitivity cannot be determined in vitro, that there is no difference in biochemical sensitivity, or that 5-fluorouracil exerts its antitumor effect by some mechanism not explored in this study.

It might be postulated that chemotherapy destroys similar numbers of tumor and normal cells but that the measurability of 3-dimensional lesions allows better quantification of cell loss in tumors than is possible in organs such as the colon. Also, the anatomic blood supply of normal tissue may permit rapid cell regeneration after cessation of therapy. In contrast, tumor tissue is supplied by tenuous vascularity which penetrates the tumor and which may be profoundly disturbed by the death of the supporting tumor. Tumor regeneration may therefore be slower than normal tissue regeneration, and this could explain the regression of tumors in some patients. Although this study does not undertake to explore such a theory, it does indicate the need for hypotheses alternative to that of preferential tumor sensitivity to 5-fluorouracil.

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REFERENCES

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