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Comparison of 5-FU versus FUDR activity in human colorectal cancer using an in vitro clonogenic assay (HTCA)

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Summary. Comparative in vitro drug testing was performed in 72 of 183 surgically removed human colorectal cancer specimens (34 primary lesions, 38 metastases). In 10 of these tumors, comparative dose-response curves were obtained. Given a $\geq 70\%$ ICF (inhibition of colony formation) as threshold for in vitro sensitivity, 5-FU was active in 16/62 specimens, and FUDR in 14/62. Significantly discordant sensitivity results were observed in 8/62 tests, 5-FU being the more active agent in 5 of these cases. These data are supported by the finding of 3 considerably differing dose-response curves in 10 additional comparative studies of human primary tumors.

Introduction

During recent years the HTCA (human tumor colony assay) has been developed by Hamburger and Salmon as a promising technique for drug testing and biological studies on primary human tumor cells. This is of particular value for those tumor types with high drug resistance, such as most gastrointestinal carcinomas.

5-FU and 5-FUDR are some of the most active compounds for chemotherapy of colorectal cancer. In clinical studies no consistent differences in activity were observed [3]. However, in some animal-derived tumor lines, which allow direct comparison, certain differences in biochemical action and antitumor effect were noted [11]. Whether this phenomenon of only partial cross-reactivity also pertains to human cancer is unknown.

This study uses the HTCA to perform in vitro comparative drug testing on primary human tumor cells to address this question.

Materials and methods

In total, 183 fresh surgical specimens of colorectal cancer were disaggregated mechanically and then assayed for drug sensitivity after a 1-h incubation and cultured according to the method described by Hamburger et al. [6], except for the omission of DEAE-dextran and mercaptoethanol. The serum concentrations used in the experiments were as follows: 10% heat-inactivated FCS and 5% horse serum in the underlayer and 15% horse serum in the upper plating layer (serum distributed by Flow Laboratories, Calif). The drug concentrations during the 1-h incubation were 60 μg 5-FU/ml and 10 μg FUDR/ml when only single doses were tested. The dose ratio between 5-FU and FUDR was chosen to give equivalent inhibition of colony formation in the human colon cancer-derived tissue culture line Wi/Dr (supplied by EORTC/CASSG) and was confirmed by comparative dose-response studies in colorectal primary tumors.

After drug exposure at a concentration of 10^6 cells/ml, the cells were washed and incubated for 14–21 days at 37 °C, 5% CO_2, and 95% humidity. The concentration was 5×10^5 cells/plate. Each experiment was done in triplicate. Colony counting was accomplished with an inverted light microscope. Aggregates over 60 μm in size were considered to be colonies. Evaluation of an assay required growth of at least 25 tumor colonies/control plate and less than 10% of equivalent aggregates in fixed day 0 controls. The definition of in vitro drug sensitivity was set at more than 70% inhibition of colony formation (ICF).

For statistical evaluation one-step regression analysis and chi-square analysis were used.

Results

Comparative drug testing could be performed in 72/183 tumor specimens of colorectal cancer with sufficient colony growth. There were 69 tumors that did not show colony growth at all, and 32 assays were lost owing to contamination. Plating efficiencies of the tumors with in vitro colony formation ranged between 0.001 and 0.41, with a mean of 0.03. Colony counts/plate in the tumors evaluated were between 27 and 193. The variation within the triplicate plates was consistently less than 20%.

Figure 1 shows comparative dose-response curves for 5-FU and FUDR in ten human colorectal cancers. In three cases considerably different slopes are seen, suggesting lack of cross-sensitivity. For quantitation D_0 values (drug concentration giving a surviving fraction of 0.37 in the steep portion of the dose survival curve) were estimated when an exponential dose-effect ratio could be assumed. From the remaining seven curves the approximate isoeffect-dose ratio between 5-FU and FUDR was 6:1. The same dose ratio was found in experiments with human colon cancer-derived tissue line Wi/Dr. According to these results, doses of 60 μg/ml for 5-FU and 10 μg/ml for FUDR were chosen for further comparative experiments at a single dose level. The results are shown in Fig. 2. Most

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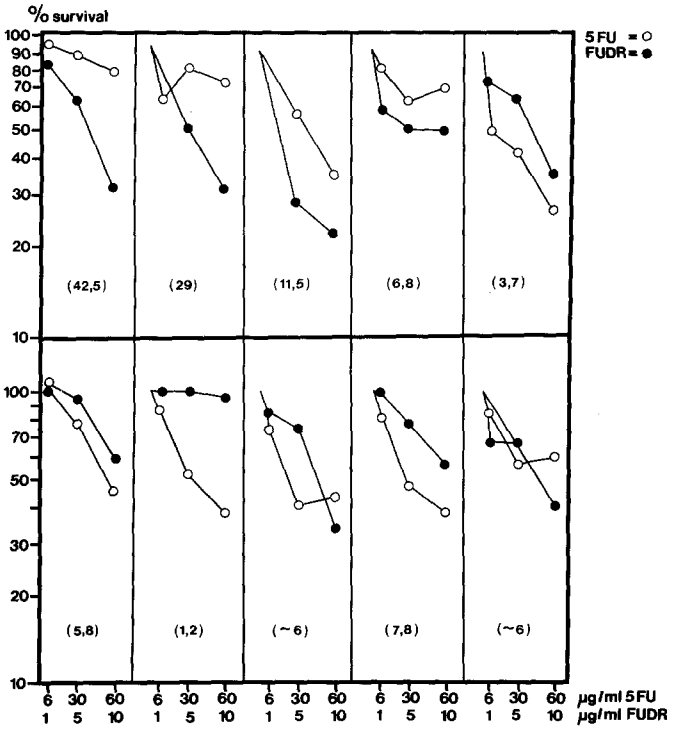

Fig. 1. Dose response comparison for 5-FU *(open symbols)* and FUDR *(closed symbols)* in human colorectal carcinomas. In brackets:

$$\text{isoeffect dose ratio} = \frac{D_0\,\text{FUDR}}{D_0\,\text{5-FU}}$$

Tumors 1, 2, and 7 show considerable differences in the slope of the survival curves against 5-FU and FUDR

of the colorectal tumors studied show good correlation of sensitivity between the two drugs (coefficient of correlation 0.62). Differing sensitivities were assumed when one drug led to $\geq 70\%$ ICF and the other $\leq 50\%$.

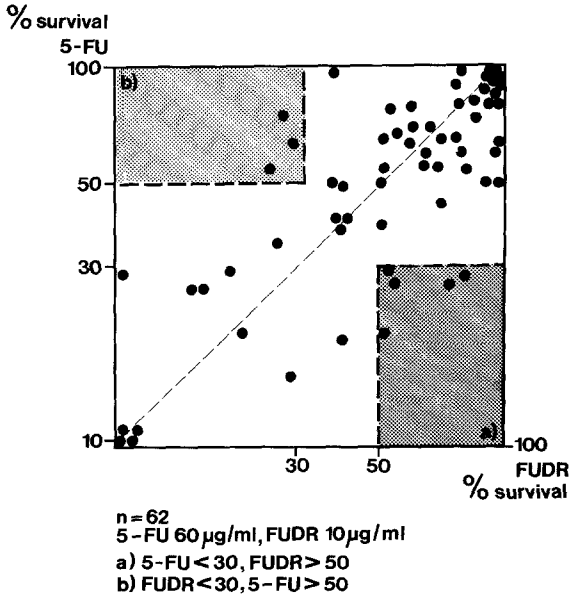

Fig. 2. In vitro comparison between 5-FU (60 µg/ml) and FUDR (10 µg/ml) in 62 human colorectal carcinomas. *Dark areas* represent discordant sensitivity between the two drugs

Using these rigid criteria, which relate to experience with in vitro-in vivo correlations [4], lack of cross-reactivity was found in 8/62 studies. In 5 of these 5-FU was superior, while in the remaining 3 tumors FUDR was the more potent drug.

Nevertheless, these criteria may even underestimate the actual proportional reduction in cross-reactivity compared with the difference in ICF between 5-FU and FUDR for each tumor or the slope of dose-response curves, which were plotted for 10 tumors.

In 14 of the 72 tumors studied, clinical correlations to the in vitro response were obtained (11 for 5-FU, 3 for FUDR). Using WHO criteria [12] for clinical response there were 4 true-positives, 6 true-negatives, 2 false-positives, and 2 false-negatives, resulting in an overall predictivity of 71.5% in this set of tumors.

Discussion

5-FU is one of the most active agents used in the treatment of colorectal cancer, with clinical response rates of 20%–25%. Similar results have been obtained in clinical studies with FUDR [2, 13]. This agent is now mainly used for regional chemotherapy of hepatic metastases on account of its nearly complete clearance by the liver [5].

The use of HTCA makes a direct comparison of drug effects against the same human tumor specimen possible, as was recently shown for adriamycin and esorubicin by Salmon et al. [16].

In our study comparative drug evaluation of 5-FU and FUDR in HTCA showed a good correlation of sensitivity at a dose ratio of 6:1 in the human colon cancer-derived tissue culture line Wi/Dr in 7 of 10 dose-response studies of surgically removed human colorectal cancer and in 54 of 62 specimens tested at a single dose level. Experiments with animal tumors and tissue culture lines have revealed a considerably lower dose of FUDR for equivalent cytotoxicity [7]. This difference might be explained by biochemical differences between the rapidly proliferating experimental tumors and primary human solid tumors. The effect could also be influenced by the specific culture conditions of the soft agar assay and the tissue culture medium used.

Antimetabolites may be particularly susceptible to those factors. However, the fairly good predictivity of in vitro-in vivo correlations in this set of tumors points to the validity of the system for at least short-term response and is in agreement with the overall results reported in the literature [1, 18].

There is good evidence that 5-FU exerts its cytotoxic effect both by inhibition of thymidylate synthetase and by incorporation into RNA [11], while FUDR is thought to inhibit mainly de novo synthesis of thymidylate, since only single-step conversion to the active metabolite FdUMP is needed. Some differences in activity between the two agents have been observed in tumor lines. The effects of FUDR were reversed by thymidine, while in some lines there was only partial thymidine rescue for 5-FU or none at all [17]. Different mechanisms of resistance to fluoropyrimidines have been described. It seems conceivable that lack of thymidine kinase [14], uridine kinase [15], or an altered thymidilate synthetase [7] may have different effects on the incorporation of fluoropyrimidines into RNA or on the inhibition of thymidylate synthesis. Moreover, a potent salvage pathway such as is described in solid tumors [19]

will mainly counterbalance the inhibitory effects on thymidylate synthesis.

Incorporation of 5-FU into RNA seems to vary widely in different tumor lines [9], and the impact of FU-RNA formation on cytotoxicity seems to differ [8, 10]. In view of the experimental evidence for possible differences in activity between 5-FU and FUDR, the lack of correlation in 3/10 dose-response studies and in 8/62 single-dose studies in this investigation suggests that there may be different biochemical targets for the action of fluoropyrimidines in human colorectal tumors. This could account for the partial lack of cross-reactivity seen.

References

1. Alberts DS, Surwit EA, Leigh S, Moon TE, Salmon SE (1982) Improved survival for relapsing ovarian cancer patients using the human tumor stem cell assay to select chemotherapy. Stem Cells 1: 294
2. Ansfield FJ, Cuerri AR (1963) Further clinical comparison between 5-fluorouracil and 5-fluoro-2-deoxyuridine. Cancer Chemother Rep 32: 101
3. Bedikian AY, Bodey GP, Valdivieso M, Stroehlein JR, Karlin DA, Bennets RW, Patt YZ, Burgess MA (1981) Chemotherapeutic management of colorectal cancer and prospects for new agents. In: Stroehlein JR, Romsdahl MM (eds) Gastrointestinal cancer. Raven, New York, p 365
4. Bertelsen CA, Sondak VK, Mann BD, Korn EL, Kern DH (1984) Chemosensitivity testing of human solid tumors. Cancer 53: 1240
5. Ensminger WD, Rosowsky A, Raso V (1978) A clinical pharmacological evaluation of hepatic arterial infusion of 5-fluoro-2-deoxyuridine and 5-fluorouracil. Cancer Res 38: 3784
6. Hamburger AW, Salmon SE, Kim MB, Trent JM, Soehnlen BJ, Alberts DS, Schmidt HJ (1978) Direct cloning of human ovarian carcinoma cells in soft agar. Cancer Res 38: 3438
7. Heidelberger C, Kaldor G, Mukherjee IL, Danneberg PB (1960) Studies on fluorinated pyrimidines: XI. In vitro studies on tumor resistance. Cancer Res 20: 903
8. Houghton PJ, Houghton JA (1978) Evaluation of single agent chemotherapy in human colorectal tumor xenografts. Br J Cancer 37: 833
9. Kessel D, Hall TC, Reyes P (1969) Metabolism of uracil and 5-fluorouracil in P388 murine leukemia cells. Mol Pharmacol 5: 481
10. Laskin JD, Evans RM, Slocum HK (1979) Basis of natural variation in sensitivity to 5-fluorouracil in mouse and human cells in culture. Cancer Res 39: 383
11. Mandel HG (1981) The target cell determinants of the antitumor actions of 5-FU. Does 5-FU incorporation into RNA play a role? Cancer Treat Rep 65 [Suppl 3]: 63
12. Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207
13. Moertel CG (1978) Current concepts in cancer chemotherapy of gastrointestinal cancer. N Engl J Med 299: 1049
14. Morse PA, Potter VR (1965) Pyrimidine metabolism in tissue culture cells derived from rat hepatomas: 1. Suspension cultures derived from the Novikoff hepatoma. Cancer Res 25: 499
15. Reichard P, Skould O, Klein G, Revesz L, Magnusson PH (1962) Studies on resistance of 5-fluorouracil: 1. Enzymes of the uracil pathway during development of resistance. Cancer Res 22: 235
16. Salmon SE, Young L, Soehnlen B, Liu R (1984) Antitumor activity of esorubicin in human tumor clonogenic assay with comparisons to doxorubicin. J Clin Oncol 2: 282
17. Umeda M, Heidelberger C (1968) Comparative studies of fluorinated pyrimidines with various cell lines. Cancer Res 28: 2529
18. Von Hoff DD, Clark GM, Stogdill BJ, Sarosdy MF, O'Brien I, Casper JZ, Mattox TE, Page GP, Cruz AB, Sandbach JF (1983) Prospective clinical trial of a human tumor cloning system. Cancer Res 43: 1926
19. Weber G (1983) Biochemical strategy of cancer cells and the design of chemotherapy. Cancer Res 43: 1019

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