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CONCENTRATION-TIME-DEPENDANT TOXICITY OF 5-FU, (F), 5-FUR (FR), and 5-FUDR (FDR) IN COLORECTAL CARCINOMA CELL LINE HT-29.

K.H.Link, K.R.Aigner, and C.Peschau.

F and FDR are used for regionalchemotherapy of colorectal liver metastases. Therapeutic concepts are differing concerning time and dosage of drug exposure. We determined the optimal concentration x time products for F, FR, and FDR to find out the most active anabolite, compared with the mother substance.
HT-29 was incubated with the drugs at various times and concentrations. The drug effect was determined by measuring the extent and duration of growth delay in soft agar culture medium. Incubation periods were 5min - 24h and doses ranged between .01 and 1000 µg/ml. The cultures were evaluated on day 6 after seeding to calculate inhibition of growth, and on days 16 and 20 to check for regrowth.
In all experiments, there was a clear dose response with significant differences in toxicity within the 3 test substances. FR was the most active drug regarding cytostatic (growth delay) and cytotoxic (duration of growth delay, lack of regrowth) activity, followed by F and FDR (1h-24h exposure: FR $\gg$ F $>$ FDR; $\leq$20min exposure: FR $\gg$ FDR $>$ F). There was a resistant subpopulation to FDR. - The differences became more pronounced at increasing doses.
In conclusion, with regard to hepatotoxicity, F should be used for hepatic artrial infusion, especially if high doses at short times can be applied. By treating with FDR, inhibition of DNA-synthesis is the major principle of action, while the other potent mechanism, formation of wrong m-RNA, is neglected.

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DOSE RESPONSE OF MITOXANTRONE IN CELL LINES AND HUMAN SOLID TUMOR BIOPSIES WITH FIRST RESULTS IN REGIONAL CHEMOTHERAPY.

K.H.Link, D.Kessler, and K.R.Aigner.

Mitoxantrone (Novantron^{R}, MX) has been shown to be effective in phase II trials in breast cancer. The response of other solid tumors to systemic chemotherapy with MX was rather poor. Due to the the drug's potency to induce dose-dependent cytotoxicity in cellular in vitro systems, we were interested whether response of solid tumors other than breast carcinoma could be improved by dose increase.
Two human tumor cell lines (Colorectal HT-29 and melanoma M-19) and our nude mouse colorectal transplant tumor NMG-64 were studied in their dose response behaviour to MX in a method related to the 'Human Tumor Colony-forming Assay'(HTCA). In Addition, 6 human tumor biopsies (1 Liposarcoma, 1 Schwannoma, 1 Adenocarcinoma, 1 Melanoma, 2 Colorectal Carcinomas) were tested in the HTCA with a wide dose range of MX. The test results were compared with the in vitro responses to drugs established for regional chemotherapy.
MX showed a clear dose response in HT-29, M-19, and NMG-64. A marked dose response was seen in 4/6 human solid tumors. In the cell lines, response was induced between .01 - 0.1 µg/ml, in the human tumor biopsies in the range of 1 µg/ml. This dose range can be clinically achieved by hepatic- and bronchial artery infusions, and intraperitoneal instillations. Our first results in regional chemotherapy with MX seem to correlate well with the experience made up to date in vitro

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Part of this work thesis from D.Kessler.

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DIFFERENCES IN THE IN VITRO-ACTIVITY OF 5-FU and FUDR AGAINST HUMAN COLORECTAL CARCINOMAS.

D.Flentje, M.Flentje, P.Schlag

F-FU and FUDR are some of the most active compounds for chemotherapy of colorectal cancer. In clinical studies, no consistent differences in activity were observed. However, in some animal derived tumor lines, which allow direct comparison, certain differences in biochemical action and anti-tumor effect were noted. Whether this phenomenon of only partial cross-reactivity also pertains to human gastrointestinal cancer is unkown.
Using an in vitro soft agar culture system (Human Tumor Colony Assay = HTCA), effects of 5-FU and FUDR were compared in 72 primary colorectal cancer specimens. A drug was considered active against the respective tumor tested, if more than 70 percent inhibition of colony growth (IGF) was obtained at single dose levels, e.g.
1/10 of the peak plasma level. Discordance of in vitro activity was assumed, when one drug resulted in an IGF over 70 while the other drug gave less than 50 percent inhibition.
62 single dose comparisons and 10 dose response curves were analyzed. In the majority of studies an isodose ratio of 6:1 between 5-FU and FUDR was found. There were 8 significantly discordant results of in vitro sensitivity in the single dose comparisons and 3 out of 10 considerably differing dose-response-curves. 5-FU was more active than FUDR in 7 cases and FUDR was more potent in 4 cases respectively. Overall, 5-FU was active (IGF over 70 %) in 18/72 experiments, FUDR in 16/72 tests.
This points to a partial lack of cross-sensitivity between 5-FU and FUDR in human colorectal cancer. Differing biochemical targets (thymidilate synthetase, RNA-incorporation) for these two drug analogues have been described in experimental tumors and may also account for the effects observed in this study.

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ENHANCED INCORPORATION OF 5-FLUOROURACIL (5-FU) INTO DNA OF A HUMAN COLON CARCINOMA AFTER PRETREATMENT WITH METHOTREXATE (MTX).

P. Dias Wickramanayake and H.O. Klein

Cadman and Bertino had shown that a sequential administration of MTX and 5-FU acts synergistically with respect to therapy of transplantable ascites and solid tumors in mice. MTX inhibits dihydrofolate reductase, and, consequently, increases the concentration of phosphoribosylpyrophosphate (PRPP). PRPP enhances the intracellular concentration of 5-FU if the time interval between MTX and 5-FU is longer than 1 hour. 5-FU is converted either to 5-FUTP which is incorporaed into RNA or to 5-FdUMP. 5-FdUMP blocks the enzyme thymidylate synthetase. Normally, 80% of 5-FU is incorporated into RNA. The aim of our study was to prove (1) whether after MTX treatment variations of PRPP concentrations in cells of two different human colon carcinomas growing on nude mice can be observed, (2) whether after MTX an increased conversion of 5-FU to 5-FdUMP occurs in vivo.
Results: 1.) Both human colon carcinomas showed increased PRPP concentrations after MTX. The process is time- and dose-dependent. Peak concentration is reached by a dose of 100 mg/kg MTX and after a time of 3,5 and 16 hours, respectively. Bone marrow cells do not show any variations of PRPP concentrations after MTX. 2.) After MTX the conversion of 5-FU to 5-FdUMP increases 9,75 fold compared with monotherapy of 5-FU, the incorporation of 5-FUTP in RNA increases 1,6 fold. The process is time-dependent. Peak concentration is reached 6 hours after MTX.
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