ISOLATED LIVER PERFUSION VERSUS HEPATIC ARTERY INFUSION WITH 5-FLUOROURACIL IN A RAT MODEL; EFFECTS ON THYMIDYLATE SYNTHASE

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INTRODUCTION

5-Fluorouracil (5FU) is frequently used for locoregional chemotherapy of non-resectable hepatic metastases of advanced colorectal cancer [1,2]. This drug is metabolized to FdUMP, a potent inhibitor of thymidylate synthase (TS), an essential enzyme in the pyrimidine de novo synthesis. Inhibition of TS leads to a decreased DNA synthesis [3].

Regional 5FU administration into the liver allows the use of a higher 5FU dose than systemic therapy. Most of the 5FU is metabolized in the liver and the concentration in the systemic circulation is lower compared to peripheral intravenous infusion. This circumnvents toxic side effects of the high dose 5FU in other tissues [1,2]. The route, usually applied for this therapy, is hepatic artery infusion (HAI) with an implanted pump, which delivers the drug into the hepatic artery [4]. Isolated liver perfusion (ILP) technique has been developed to selectively expose the liver to higher doses of chemotherapy, while systemic toxicity is minimal. This method is still experimental and with 5FU clinical succes is limited [5].

We studied the biochemical effects of both ways of regional 5FU administration in the rat by evaluating the extent and retention of TS inhibition in tumor and normal tissues, in which toxic side effects of 5FU might occur.

METHODS

Hepatic metastases of the rat colon tumor line CC531 were evoked in WAG/Rij rats by subcapsular injection of viable tumor cells into the liver. Two weeks after inocculation of the tumor cells, the animals received a locoregional treatment with 5FU. 5FU was administrered at its maximally tolerated dose (MTD) either by ILP (150 mg/kg) or by HAI (50 mg/kg). For ILP, the pyloric vein, the gastroduodenal artery and the intrahepatic caval vein were cannulated.

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During perfusion the caval vein was clamped above and below the liver, the aorta proximal to the coeliac axis and the portal vein and common hepatic artery were clamped just below the tips of the two cannulas. Two low-flow roller pumps and an adapted oxygenator were used to recirculate the perfusate, containing 150 mg/kg 5FU, during 20 min [6]. For HAI, the gastroduodenal artery side arm of the common hepatic artery was cannulated and 50 mg/kg 5FU was administered [6].

Tumors, liver tissue and bone marrow were removed 3, 24 or 48 hr after treatment and immediately frozen in liquid nitrogen. Samples from untreated rats served as controls. TS inhibition was evaluated with the FdUMP binding assay, a ligand binding assay with [6-^{3}H]-FdUMP, used to determine the free binding sites of the enzyme for FdUMP, performed essentially according to published methods [7-9]. Cytosols of samples from treated rats were washed with neutral charcoal before the assay in order to remove endogenous nucleotides. Assays were performed with saturating folate concentrations to allow maximal binding of FdUMP [10].

RESULTS

The activity of TS in tissues from untreated rats was higher in tumors than in normal livers (34 vs 9 pmol/g wet weight). Also other normal tissues (intestinal mucosa and bone marrow) had a lower TS activity than tumor tissue. Treatment with 5FU resulted in a marked inhibition of TS in tumor tissue after 3 hr (Fig 1). In tumors from animals treated by HAI, recovery of TS activity was observed after 24 hr, while in tumors from rats treated by ILP, TS inhibition was prolonged. A gradual recovery of TS activity was observed for ILP tumors after 48 hr.

In liver tissue no significant inhibition of TS was observed for either route of administration (Fig 1). After 3 hr FdUMP binding to TS was comparable to control, but after 24 and 48 hr an increase of FdUMP binding was detected. This consequent increase of TS activity was most pronounced in liver tissue of rats treated by ILP. In bone marrow we observed a strong inhibition of TS 3 hr after HAI (Fig 1). Retention of TS inhibition in bone marrow was at least 48 hr in rats treated by HAI and the inhibition of TS was higher compared to bone marrow from rats treated by ILP. The latter showed a recovery of TS inhibition after 48 hr.

DISCUSSION

Biochemical analysis of tumors after ILP or HAI delivery of 5FU showed a difference in the effect on TS activity. This might have consequences for the antitumor effect of both treatments. TS activity was higher in tumor tissues compared to other highly proliferative tissues such as intestinal mucosa and bone marrow. It has been shown that TS activity is high in another proliferative tissue such as regenerating liver [11]. The difference in TS activity between hepatic metastases and normal liver tissues was about 3.5 fold. This was comparable to results of Berne et al. [12] in another rat colon tumor model.

Evaluation of TS inhibition in tumors after treatment with 5FU showed a more pronounced effect for ILP. This can partially be explained by the higher MTD of

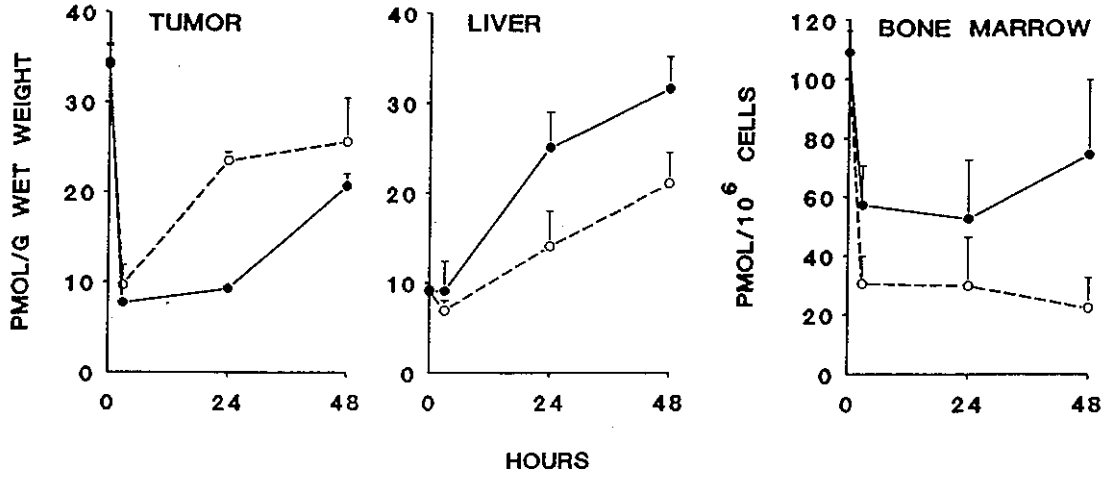

Figure 1. Comparison of the residual binding sites for [^{6}H]-FdUMP of TS after ILP (150 mg/kg 5FU) [-●-] and HAI (50 mg/kg 5FU) [-o-] in CC531 tumors, liver tissue and bone marrow of WAG/Rij rats. Values are means ± SEM, n=3-5.

5FU, that can be used for ILP (150 vs 50) and the exposure of the metastases to a higher 5FU concentration during the perfusion [13]. A slightly better antitumor effect, although not significantly different, was observed for ILP. This might correlate with the greater TS inhibition of ILP. Retention of TS inhibition in tumors was longer for ILP than for HAI. Measurements of FdUMP binding to TS in subcutaneous implanted rat colon tumors after i.p. administration of 100 mg 5FU/kg performed by others [7,12], showed that TS was completely inhibited up to 4 hr. This might be related to a higher sensitivity of the tumors to TS inhibition or a lower sensitivity of the assay.

ILP did not cause liver toxicity at this dose, this was supported by the absence of TS inhibition in the liver. For liver tissue we observed an increase of TS, probably in response to cytotoxic stress caused by 5FU exposure which can synchronize cells into S-fase [14]. It has been shown that TS activity is cell cycle dependent with a higher TS activity in the S-phase [14,15]. New enzyme synthesis may also be responsible for the increase of TS [15]. An increase of TS has been observed in rat and murine tumors shortly after i.p. 5FU treatment [7,8,12]. This phenomenom might be a resistance mechanism to 5FU. HAI resulted in a stronger TS inhibition in bone marrow compared to ILP. This more severe toxicity might be related to higher systemic exposure of normal tissues to 5FU, since plasma 5FU levels were significantly higher for HAI compared to ILP [13]. Nevertheless dose limiting toxicity for ILP is still systemic, and not liver toxicity.

These data show that ILP resulted in a better retention of TS inhibition in tumors than HAI, while HAI resulted in stronger TS inhibition in normal tissues. This points to a better therapeutic efficacy of ILP with selective exposure of the tumor and low systemic toxicity. However, ILP is a rather complicated surgical technique with several drawbacks. The initial extent of TS inhibition does not seem to be dose dependent, but TS inhibition may be prolonged by a longer administration period. So both HAI and ILP may be improved by using a longer

infusion period of the drug or by combination of drugs such as leucovorin, which can prolong TS inhibition [16,17]. Systemic toxicity, such as leukopenia may be regulated by the use of a protecting agent such as uridine [18].

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