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High mitomycin C concentration in tumour tissue can be achieved by isolated liver perfusion in rats*

Andreas Marinelli[1], Dirk H. A. Pons[1], Jacqueline A. C. Vreeken[3], Suresh K. Nagesser[1], Peter J. K. Kuppen[2], Ubbo R. Tjaden[3], and Cornelis J. H. van de Velde[1,*]

Departments of [1] Surgery, [2] Pathology, University Hospital, P. O. Box 9600, 2300 RC Leiden,
[3] Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, University, Leiden, The Netherlands

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Summary. To enable the treatment of hepatic metastasis with higher, theoretically more effective, doses of systemically toxic anticancer drugs, an isolated liver perfusion (ILP) technique was developed in WAG/Ola rats. First, in a toxicity study the maximally tolerated dose (MTD) of mitomycin C (MMC) was determined for a 25-min ILP and for hepatic artery infusion (HAI) after the administration of a bolus dose. The MTD in the ILP setting (4.8 mg/kg) was 4 times that using HAI (1.2 mg/kg). Subsequently, in a rat colorectal hepatic-metastasis model, concentrations of MMC in tumour, liver, plasma and perfusate were measured during a 25-min ILP to investigate the expected pharmacokinetic advantage of ILP. The mean plasma level determined after ILP (1.2 as well as 4.8 mg/kg MMC) was significantly lower ($P < 0.001$) than that obtained following HAI. This may explain both the absence of severe systemic toxicity and the higher MTD in ILP-treated groups. No significant difference in mean tumour and liver tissue concentrations of MMC were found when the groups treated with 1.2 mg/kg drug via HAI vs ILP were compared. The mean MMC concentration in tumour tissue was significantly higher (almost 5 times; $P < 0.05$) in rats treated by ILP with the MTD (4.8 mg/kg) than in those treated via HAI with the MTD (1.2 mg/kg). ILP of MMC can be safely performed using a dose 4 times higher than the MTD in the HAI setting, leading to an almost 5-fold concentration of MMC in hepatic metastasis. ILP of MMC may therefore represent a promising therapy for metastasis confined to the liver.

Introduction

To improve chemotherapeutic efficacy, regional approaches have been devised in the treatment of hepatic metastasis [2, 7, 18]. The advantage of regional over systemic administration is the ability to generate higher local drug concentrations while maintaining lower systemic levels [4].

Since the most common source of hepatic metastasis is colorectal cancer, we studied isolated liver perfusion (ILP) in a rat colorectal cancer model. Mitomycin C (MMC) was chosen because it is one of the few drugs that produces some tumour response in the treatment of colorectal cancer [5]. High drug concentrations are attractive since the antitumour activity of mitomycin C shows a steep dose-response curve [6, 8]; however, severe side effects prohibit dose increase during systemic administration [10, 13, 14, 16, 17, 22, 23].

In the present study, the maximally tolerated doses of MMC in the ILP setting and for hepatic artery infusion (HAI) were determined by evaluating survival and weight loss. To determine whether a higher dose of MMC in ILP would result in higher concentrations of the drug in tumour tissue, we measured tumour drug levels using high-performance liquid chromatography (HPLC) [21]. Rats were treated with the MTD for HAI or with that for ILP. Biopsies were taken from the liver and tumour, and perfusate samples were also taken from ILP-treated rats. The entire study was performed in a rat colorectal hepatic-metastasis model.

Materials and methods

Animals. Inbred WAG/Ola rats (Harlan/Olac; C. P. B., Zeist, The Netherlands) that weighed 320–400 g before determinations of the MTD of MMC in HAI and ILP and 250–300 g throughout the pharmacokinetic study were used.

Mitomycin C. MMC was dissolved in ≥2 ml 0.9% NaCl to a maximal concentration of 0.5 mg MMC/ml, which is the prescribed maximal concentration in which MMC should be dissolved. To reduce the infu-

Abbreviations: HAI, hepatic artery infusion; HPLC, high-performance liquid chromatography; ILP, isolated liver perfusion; MMC, mitomycin C; MTD, maximally tolerated dose

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Offprint requests to: C. J. H. van de Velde

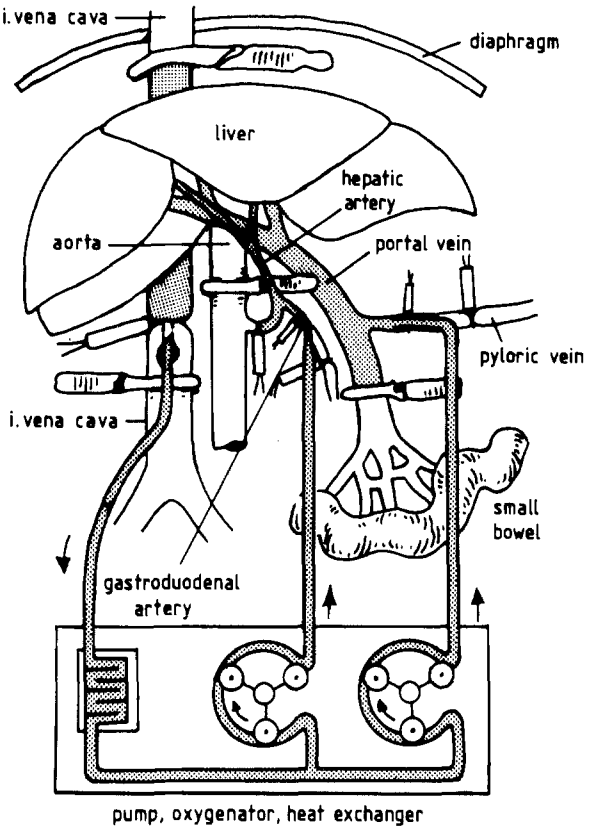

Fig. 1. Isolated liver perfusion circuit in the WAG rat

sion volume to a maximum of 2.5 ml, we dissolved MMC at a higher concentration (maximally 0.65 mg/ml) for the rats treated with a dose of 4.8 mg/kg via HAI. These solutions were prepared shortly before infusion. No crystallization of MMC was seen. MMC was obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan).

Surgical procedures. All operative procedures were carried out under clean but not sterile conditions using an operation microscope (magnification, ×20; Applied Fiberoptics, Southbridge, Mass, USA). Anesthesia was induced and maintained using ether.

ILP technique. This technique has been described elsewhere [2]. Briefly, a midline abdominal incision was performed. The caval vein was clamped just beneath the diaphragm and just above the right renal vein (Fig. 1). The intrahepatic part of the caval vein was cannulated to drain all hepatic outflow to the oxygenator and heat exchanger. Two roller pumps reinfused the perfusate (30 ml Haemaccel; Hoechst, Amsterdam, The Netherlands), one into the gastroduodenal artery, a side arm of the hepatic artery, and the other into the pyloric vein, a side arm of the portal vein. To complete the isolation, clamps were placed on the aorta, celiac trunk and portal vein. The perfusion was carried out over 25 min, during which time the intestines were cooled at 0° C. The saline-MMC solution was added to the perfusate as a bolus at the start of the perfusion. The entire procedure took 2–2.5 h.

Hepatic artery infusion. HAI was performed via the cannulated gastroduodenal branch of the common hepatic artery. During the 2-min infusion the common hepatic artery was clamped to prevent retrograde flow into the aorta. The total operation time was 20–30 min.

Sample pretreatment. To determine the drug recovery, we spiked 2 ml mobile phase and 2 ml acetonitrile with various known amounts of MMC. Immediately after the addition of 200 mg liver to the acetonitrile solutions, the samples were pretreated for HPLC analysis as described below and subsequently injected into the HPLC system; the mobile phase solutions were directly injected. HPLC analysis of the samples revealed that the recovery of MMC from the solution containing homogenized liver was 100%.

Liver and tumour tissue were homogenized in acetonitrile (HPLC grade, 2 ml; Chemicals Limited, Walkerburn, Scotland) using a Polytron (Kinematica, Luzern, Switzerland) to stop the metabolism of MMC. The samples were frozen in liquid nitrogen and stored at –30° C in a refrigerator. Before analysis the samples were thawed and centrifuged at 6,000 rpm. An aliquot (500–1,000 µl) of the supernatant was dried in the vacuum centrifuge, dissolved in the mobile phase [500 µl of a mixture of 0.05 M phosphate buffer, pH 7 (85%), and acetonitrile (15%)] and then injected (100 µl) into the HPLC system. Plasma and perfusate were centrifuged for 15 min at 2,000 rpm. The supernatant was removed and stored at –30° C. Prior to analysis, the plasma and perfusate supernatants were thawed, centrifuged at 6,000 rpm, diluted with water and injected (100 µl) directly into the HPLC.

Together with the samples from each rat, samples were collected for a calibration line; various known amounts of MMC were added to 2 ml acetonitrile with 200 mg liver. The pretreatment and storage of these calibration-line samples were identical with those used for the samples taken from rats (see above). HPLC analysis of calibration samples and "rat" samples was always done on the same day.

Drug analysis. All MMC concentrations were measured using HPLC [22]. The HPLC system consisted of a high-pressure pump (Familic 300s; Jasco, Tokyo, Japan) combined with an LC-UV variable-wavelength detector (Spectroflow 773; Kratos, Ramsey, N. J., USA). Samples were injected using a Promis autosampler (LKB, Bromma, Sweden), and integration was done by a model C-R3A integrator (Shimadzu, Kyoto, Japan). The column was a stainless steel tube (100 mm in length and 3 mm in inside diameter) packed with Nucleosil C8 µm particles (Macherey-Nagel, Düren, FRG). The flow-rate was 0.5 ml/min, and the UV detector was set at 360 nm.

Tumour model. The syngeneic, weakly immunogenic tumour line CC531 is a dimethyl hydrazine-induced carcinoma of the colon [15]. From an established tissue-culture cell line, exponentially growing cells were trypsinized, washed and diluted with Hanks' balanced salt solution (University Hospital, Leiden, The Netherlands) to a volume of 10^7 cells/ml. Three hepatic tumours were induced by subcapsular injection of 0.5×10^6 cells/0.05 ml in three different liver lobes: the right main lobe, the right accessory lobe and the left main lobe. At 10 days post-injection, the average cross-sectional area of the tumour was $0.25 \times \pi \times 6 \times 6$ mm^2.

Design of the toxicity study. First, the MTD of MMC for HAI was determined by assigning 16 rats to 4 doses: 1.2, 2.4, 3.6 and 4.8 mg/kg. After this round, six rats were treated with 1.5 mg/kg MMC. Then, the doses for the ILP toxicity study were chosen, representing 1, 3, 4 and 5 times the MTD for HAI, respectively. Toxicity was evaluated by determinations of weight, WBC count, blood chemistry (sodium, potassium, creatinine, urea, bilirubin, serum glutamic-oxaloacetic and -pyruvic transaminase) and survival. Weight was recorded twice a week on days 3 and 7. WBC counts were determined on day 3 and then every week together with blood chemistry. The rats were killed on day 56.

Treatment groups in the pharmacokinetic study. For the pharmacokinetic study, rats were randomly assigned to 3 different groups: group 1 ($n = 5$) received 1.2 mg/kg MMC via bolus HAI, group 2 ($n = 6$) was given 1.2 mg/kg and group 3 ($n = 6$), 4.8 mg/kg in a 25-min ILP setting.

Samples for the pharmacokinetic study. Liver samples were taken at t = 0, t = 5, t = 10, t = 15 and t = 20. A sample was also taken from ILP-treated rats at t = 25 to measure the effect of the washout. Tumour samples were removed at t = 5, t = 15, and t = 20, and perfusate samples were obtained at t = 0, t = 5, t = 10, t = 15 and t = 20; a plasma sample was taken at t = 25. For all sampling, t = 0 represents time before injection of the MMC into the hepatic artery (HAI) or perfusate (ILP) and t = 25 stands for 25 min after the infusion (HAI) or just after washout (ILP). For liver tissue biopsies, part of a liver lobe was ligated (linen 60) and excised (there was no loss of either perfusate during ILP or blood during HAI). When a tumour sample was taken, part of a liver lobe containing a whole tumour was ligated and excised, and then tumour

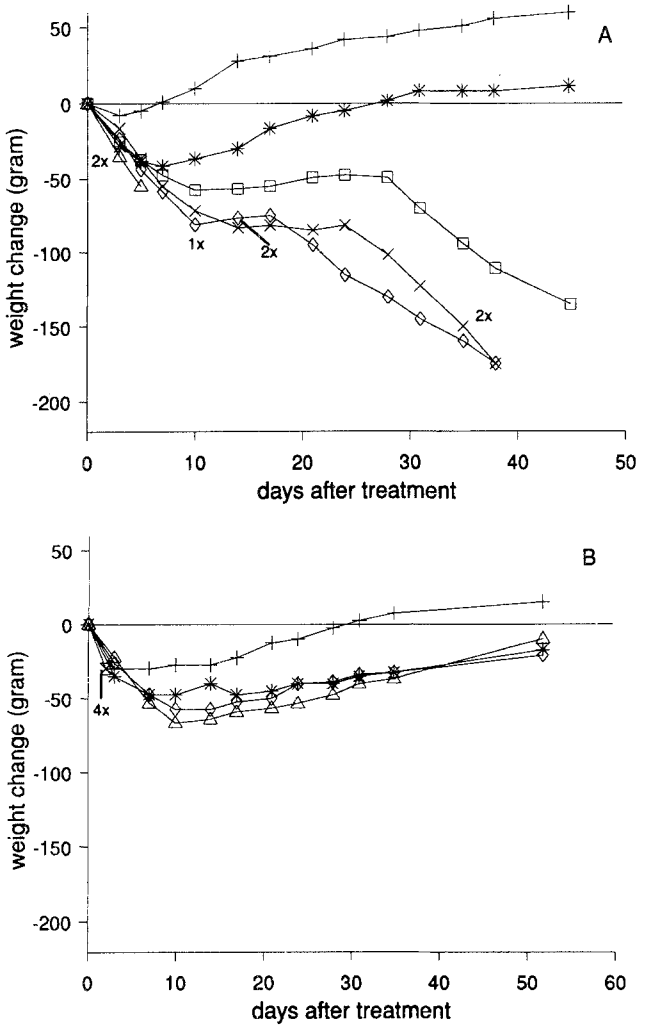

Fig. 2 A, B. Average change in the weight of WAG/Ola rats (weighing 320–400 g) treated with different doses of MMC **A** by bolus hepatic artery infusion [+, 0 ($n = 4$); *, 1.2 mg/kg ($n = 4$); ⊟, 1.5 mg/kg ($n = 6$); ⋇, 2.4 mg/kg ($n = 4$); ◇, 3.6 mg/kg ($n = 4$); △, 4.8 mg/kg ($n = 4$)] or **B** in a 25-min isolated liver perfusion setting [+, 0 ($n = 2$); *, 1.2 mg/kg ($n = 2$); ◇, 3.6 mg/kg ($n = 4$); △, 4.8 mg/kg ($n = 6$); ⋇, 6 mg/kg ($n = 4$)]. The numbers in the Figure 1×, 2×, 4× indicate the number or rats in the group that died on that day due to the toxic side effects of MMC

tissue was separated from the liver tissue. Liver samples without tumour weighed 100–150 mg. The tumour sample always included the whole tumour present in a given ligated liver lobe (tumour weight, 70–120 mg).

Statistical evaluation. Data were computerized to facilitate statistical analysis. For the dose-finding study as well as the pharmacokinetic study, multi-variable analysis of variance with repeated measurements was initially used to compare weight or concentration changes across time versus the different treatment groups. Because of the significant interaction between these factors and, hence, the complex interpretation and description of the results, one-way analysis of variance was used at each time point to compare the means of the different groups. If significant differences were detected, a Scheffe multiple-range test was performed. A P value of <0.05 was considered to be significant.

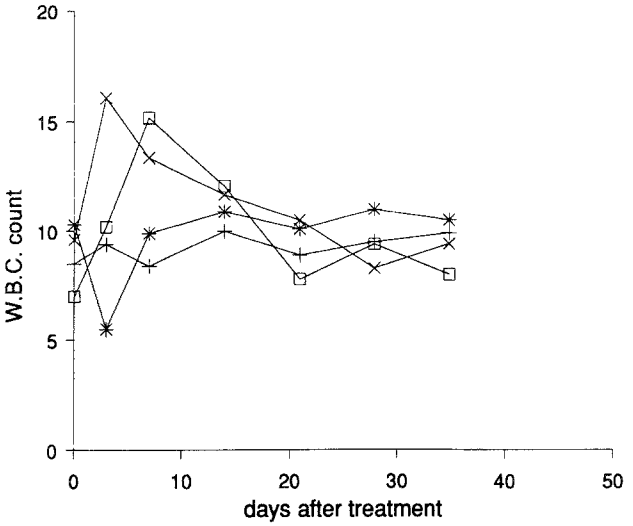

Fig. 3. Average WBC count ($\times 10^9$/l) after administration of bolus MMC via hepatic artery infusion [+, 0 ($n = 4$); *, 1.2 mg/kg ($n = 4$)] or by isolated liver perfusion [⊟, 1.2 mg/kg ($n = 2$), ⋇, 4.8 mg/kg ($n = 6$)

Results

Time-weight change curves

Hepatic artery infusion. The average change in weight curves after HAI treatment are illustrated in Fig. 2A. Following the administeration of 4.8 mg/kg MMC by HAI, acute toxicity was fatal for all rats within 5 days; these animals lost 10 g body weight/day (Fig. 2A). When the dose had been lowered to 1.5 mg/kg, three phases were distinguishable in the time-weight curves: a rapid weight loss, a steady state and a second fall in eight, which led to death (Fig. 2A).

After a bolus HAI of 1.2 mg/kg none of the rats died (Fig. 2A); these animals showed a maximal weight loss between days 8 and 12, but by about day 28 they had regained their starting weight. All rats treated with MMC via HAI exhibited a significant decrease in WBC count on day 3, but WBCs returned to normal levels within 7 days (Fig. 3). Sham HAI had no effect on the WBC count. None of the HAI-treated rats showed an increase in SGOT, SGPT or bilirubin values after treatment.

Isolated liver perfusion. In contrast to the HAI-treated animals all rats treated with ILP survived a dose of 4.8 mg/kg MMC (4 times the MTD for HAI). Rats treated with high-dose MMC exhibited a dip in their weight between days 9 and 12 (mean values, –58 g after 3.6 mg/kg and –67 g after 4.8 mg/kg). It is unlikely that all of this weight loss was due to MMC toxicity, since a sham ILP caused a mean weight loss of –27 g. The results are illustrated in Fig. 2B.

All ILP-treated rats exhibited a significant increase in WBC count on day 3 (Fig. 3), but in most cases WBCs returned to normal levels within 7 days. Like the HAI-treated rats, the animals treated with 1.2 mg/kg in the ILP setting also showed no increase in plasma levels of SGOT, SGPT or bilirubin. However, after ILP using 3.6 and 4.8 mg/kg MMC, values for SGOT, SGPT and bilirubin were increased; in all rats, the highest values were found at

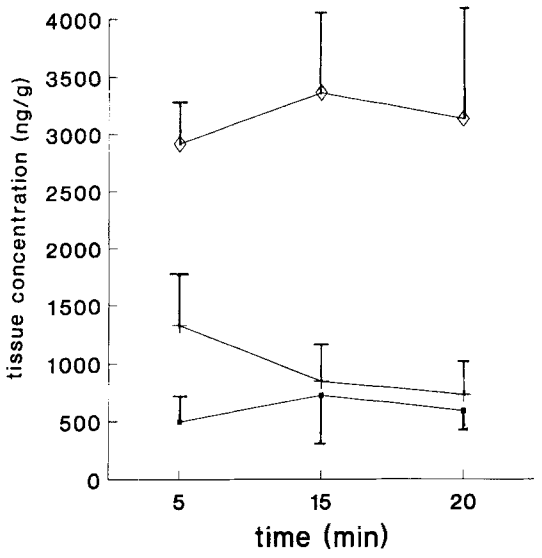

Fig. 4. Mean concentrations of MMC in tumour tissue (+ SE) at various time points in the three treatment groups: ■, 1.2 mg/kg HAI ($n = 5$); +, 1.2 mg/kg ILP ($n = 6$); ◇, 4.8 mg/kg ILP ($n = 6$)

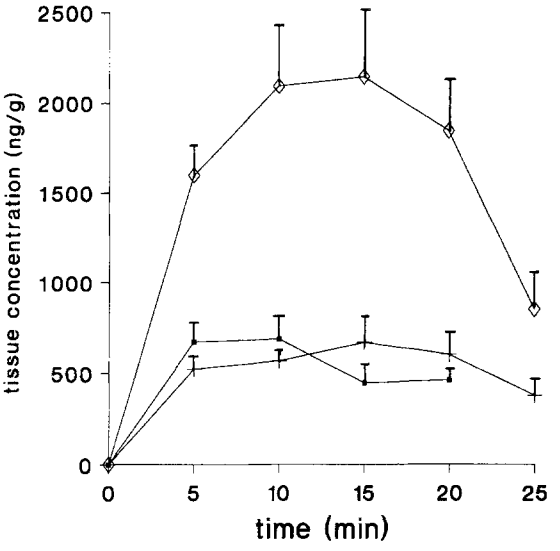

Fig. 5. Mean concentrations of MMC in liver tissue (+ SE) at various time points in the three treatment groups: ■, 1.2 mg/kg HAI ($n = 5$); +, 1.2 mg/kg ILP ($n = 6$); ◇, 4.8 mg/kg ILP ($n = 6$)

7 days after treatment (maximal levels: SGOT and SGPT, 3–4 times the pretreatment value; bilirubin, 8–9 times the preoperative level) and these levels returned to normal within 3–5 weeks.

Tissue concentrations

MMC concentrations in tumour tissue at various time points are presented in Fig. 4. Between the two groups (HAI and ILP) that received the MTD of MMC for HAI (1.2 mg/kg), no significant difference could be demonstrated. In the group that received 4.8 mg/kg, the MTD for ILP, significantly higher drug concentrations were found at all time points. The peak tumour-tissue concentration in the group receiving 4.8 mg/kg MMC was 2–3 times that found in the group given 1.2 mg/kg by ILP (1,328 vs 3,366 ng/g).

Liver-tissue concentrations of MMC (Fig. 5) also showed no significant difference between the HAI and ILP groups treated with 1.2 mg/kg MMC. Again, significantly higher levels of MMC were measured in liver tissue at all time points after ILP using 4.8 mg/kg MMC.

Perfusate levels

The MMC levels in perfusate showed a significant, continuous decrease between t = 5 and t = 20 (Fig. 6), whereas drug concentrations in liver as well as in tumour tissue either remained the same or increased until t = 15 (Figs. 4, 5).

Plasma levels

Systemic plasma levels at the end of infusion (HAI) or after the washout (ILP) were significantly ($P < 0.001$) lower after ILP [1.2 mg/kg dose, 22 ng/ml ($n = 6$); 4.8 mg/kg dose, 212 ng/ml ($n = 6$)] than after HAI (539 ng/ml, ($n = 5$)). In the HAI setting, the whole dose enters the systemic circulation except for the 10%–15% that is eliminated during first-pass metabolism, whereas in ILP, only leakage from the isolated circuit to the systemic circulation is responsible for the systemic levels.

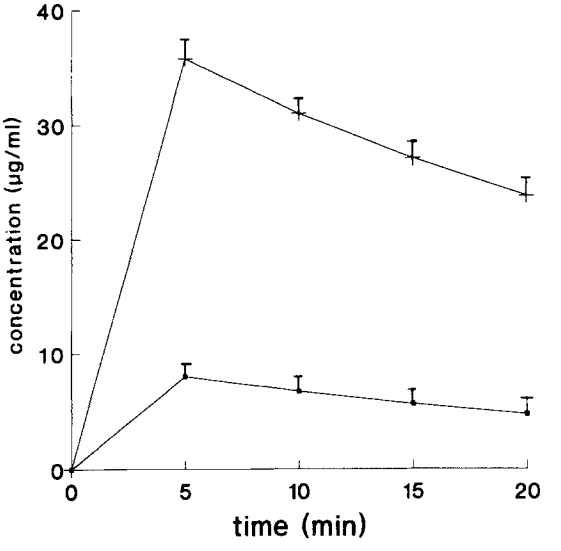

Fig. 6. Mean MMC concentrations in perfusate (+ SE) versus the time curve in the two ILP-treated groups: ■, 1.2 mg/kg ($n = 6$); +, 4.8 mg/kg ($n = 6$)

Discussion

Based on the finding that the dose-response relationship is steep for MMC [6, 8], it seems attractive to generate high local drug levels to treat hepatic metastasis. Prolonged exposure of the tumour cells to high-dose MMC could

have a beneficial effect [3, 20]. Since systemic toxicity is dose-limiting in short-term high-dose MMC treatment, isolated liver perfusion was developed to enable a further dose increase and a prolonged exposure to high concentrations of MMC.

In the present study, we compared the toxicity pattern of different MMC doses in the HAI and ILP settings. Survival, WBC count, blood chemistry and weight loss were chosen as toxicity parameters. In all HAI-treated rats, a transient bone marrow depression was observed during the 1st week after treatment, whereas in ILP-treated animals, no systemic toxicity could be detected. In ILP-treated rats, the minimal leakage of MMC to the systemic circulation prevents systemic toxicity, even following the administration of a dose that is 4 times the MTD for HAI.

The major organ involved in the metabolism of MMC the liver [12], proved to tolerate the drug very well. Even in rats treated with 4.8 mg/kg by ILP, only a transient increase in plasma levels of SGOT, SGPT and bilirubin was detectable. In the ILP setting, all animals treated with 4.8 mg/kg survived the therapy (whereas a bolus infusion of as little as 1.5 mg/kg MMC via the hepatic artery was lethal due to systemic toxicity). None of the rats treated with 4.8 mg/kg developed veno-occlusive disease of the liver, which was seen in some patients in a clinical study evaluating intensive MMC therapy and autologous bone marrow transplantation [11]. In contrast 6 mg/kg MMC was fatal to all animals within 3 days; severe, direct liver-cell toxicity led to multiple infarction and massive hepatocellular necrosis. These results clearly demonstrate that in ILP with MMC in the present rat model, liver toxicity is dose-limiting.

In this study, the ILP technique was extended by the use of a cannula in the gastroduodenal artery as a second infusion limb in the perfusion circuit. This HAI limb is essential, since established liver metastases receive most of their blood supply from the hepatic artery [1, 19]. As two inflow limbs represent be the optimal operation technique in clinical practice, we compared this ILP technique (with inflow via both the potal vein and the hepatic artery) with direct HAI.

In the pharmacokinetic study, no significant difference was found between the MMC concentrations in tumour tissue following the administration of 1.2 mg/kg by ILP or via HAI. However, plasma drug levels were lower in the ILP-treated rats. Exploiting the possiblitiy of increasing the dose 4 times for ILP in comparison with HAI based on the toxicity study resulted in significantly higher MMC concentrations in tumour tissue. The experiments showed that tumour-tissue concentrations were much higher after ILP using 4.8 mg/kg MMC than after either HAI or ILP using 1.2 mg/kg.

The MMC concentrations in perfusate decreased continuously from t = 5 until t = 20. The tumour and liver-tissue levels increased to a peak tissue concentration at t = 15; this indicates that in spite of the decreasing MMC concentrations in perfusate, MMC was being transferred into the tissues. The difference between the concentration-time curve in perfusate and that in liver and tumour suggested that the concentrations measured in liver and tumour tissue did not merely reflect intravascular MMC. Another argument for the assumption that extravascular MMC must have contributed to the concentrations measured in the biopsies is that in spite of the poor vascularisation of the tumours, the MMC levels found were the same, if not higher, in tumour tissue than in the very well-vascularised liver tissue. MMC metabolism was stopped first in liver tissue and then in tumour tissue when biopsies were taken simultaneously; thus, this difference in concentration cannot be the result of longer MMC metabolism in the liver. A third argument involves the high drug level found in liver tissue after the washout, when the intravascular compartment no longer contained MMC.

In liver tissue, the difference in MMC concentrations between the different groups was similar to that in tumour tissue. As was demonstrated in the toxicity study, liver tissue could overcome the toxic effects of these increased tissue concentrations. The questions as to whether the tissue concentration is high enough to kill the tumour cells needs further investigation. In our experiment, MMC concentrations in tumour tissue of rats treated with 4.8 mg/kg in the ILP setting reached a peak of 3,366 ng/g. Previous in vitro experiments [20, 24] have demonstrated that this concentration is sufficient to kill most tumour cells, even MMC-resistant ones. After a 1-h exposure period, Slee et al. [20] found 50% survival in a human colony-forming assay using 500 ng/ml MMC. Wilson et al. [24] showed that the resistant cell line HCT 116 R 11 (colon carcinoma line) exhibited 50% survival following exposure to 700 ng/ml MMC in a cell culture, whereas for the cell line HCT 116 R 22, 50% survival was found using 1,300 ng/ml. Assuming that the sensitivity of CC531 is comparable with that of these two tumours, extrapolation of these in vitro findings to our in vivo results suggests that the MMC levels accomplished with 4.8 mg/kg by ILP were high enough to kill most of the tumour cells.

From these experiments it can be concluded that ILP enabled the use of an MMC dose 4-fold that for HAI and that the MMC concentration increased significantly in rats treated with 4.8 mg/kg as compared with those given 1.2 mg/kg either by ILP or via bolus HAI. This increase in tumour-tissue concentration could be the difference between a cure and a partial remission of liver metastasis. The preliminary results of ILP perfusion are promising: even complete remissions were observed in ILP-treated rats but not in those that underwent HAI. Plasma concentrations after the administration of 4.8 mg/kg by ILP were lower than those following bolus HAI of 1.2 mg/kg, and these lower levels did not result in detectable systemic toxicity.

References

1. Ackerman NB (1974) The blood supply of experimental liver metatasis: IV. Changes in vascularity with increasing tumor growth. Surgery 75: 589
2. Brauw LM de, Velde CJH van de, Tjaden UR, Bruijn EA de, Bele AVRJ, Hermans J, Zwaveling A (1988) In vivo isolated liver perfusion technique in a rat hepatic metastasis model: 5-fluorouracil concentrations in tumor tissue. J Surg Res 44: 137
3. Bruijn EA de, Slee PHThJ, Kuppen PJK, Hoeven RA van der, Tjaden UR, Oosterom AT van, Velde CJH van de, Brauw LM de, Tanke HJ,

regional chemotherapy: MMC and fluoropyrimidines. Contr Oncol 29: 43
4. Crooke ST, Bradner WT (1976) Mitomycin C: a review. Cancer Treat Rev 3: 121
5. Doll DC, Weiss RB, Issell BF (1985) Mitomycin: ten years after approval for marketing. J Clin Oncol 3: 276
6. Drewinko B, Patchin M, Yang L (1981) Differential killing of twenty anti-tumor drugs on proliferating and non-proliferating human tumor cells. Cancer Res 41: 2328
7. Ensminger WD, Gyves JW (1984) Regional cancer chemotherapy. Cancer Treat Rep 68: 101
8. Frei E III, Canellos GP (1980) Dose: a critical factor in cancer chemotherapy. Am J Med 69: 584
9. Griswold DP Jr, Trader MW, Frei E III, Peters WP, Wolpert MK, Laster WR Jr (1987) Response of drug sensitive and resistant L1210 leukemia cells to high dose chemotherapy. Cancer Res 47: 2323
10. Gunstream SR, Seidenfeld JJ, Sibonya RE, McMahon LJ (1983) Mitomycin-associated lung disease. Cancer Treat Rep 67: 301
11. Kerpel-Fronius S, Verweij J, Stuurman MM, Kanyár B, Lelieveld P, Pinedo HM (1988) Pharmacokinetics and toxicity of mitomycin C in rodents given alone, in combination or after induction of microsomal drug metabolism. Cancer Chemother Pharmacol 22: 104
12. Lazarus HM, Gottfried MR, Herzig RH Phillips GL, Weiner RS, Sama GP, Fay J, Wolff SN, Sudilovsky O, Gale RP, Herzig GP (1982) Venoocclusive disease of the liver after high dose mitomycin C therapy and autologous bone marrow transplantation. Cancer 49: 1789
13. Levillian R, Cluzan R (1973) Cardiac toxicity of anti-mitotic drugs. Proceedings, 3rd Meeting of the European Association for Cancer Research, b, p 100
14. Liu K, Mittelman A, Sproul EE, Elias EG (1971) Renal toxicity in man treated with MMC. Cancer 28: 1314
15. Marquet RL, Westbroek DL, Jeekel J (1984) Interferon treatment of a transplantable rat colon carcinoma. Int J Cancer 38: 689
16. Orwoll ES, Kiessling PJ, Patterson JR (1978) Interstitial pneumonia from MMC. Ann Intern Med 89: 352
17. Philips FS, Schwartz HS, Sternberg SS (1960) Pharmacology of mitomycin C, toxicity and pathologic effects. Cancer Res 20: 1354
18. Ridge JA, Collin C, Bading JR (1988) Increased Adriamycin levels in hepatic implants of rabbit Vx-2 carcinoma from regional infusion. Cancer Res 48: 4584
19. Sigurdson ER, Ridge JA, Kemeny N, Daly JM (1986) Tumor and liver drug uptake following hepatic artery and portal vein infusion. J Clin Oncol 5: 1836
20. Slee PHThJ, Bruijn EA, Leeflang P, Kuppen PJK, Berg L van den, Oosterom AT van (1986) Variations in exposure to mitomycin C in an in vitro colony-forming assay. Br J Cancer 54: 951
21. Tjaden UR, Bruijn EA de, Hoeven RAM van der, Jol C, Greet J van der, Lingeman H (1987) Automated analysis of MMC in body fluids by high-performance liquid chromatography with on-line sample pretreatment. J Chromatogr 420: 53
22. Verweij J (1986) Studies on the toxicology of MMC. Thesis, Free University of Amsterdam, The Netherlands
23. Verweij J, Zanten T van, Souren T, Goldwig R, Pinedo HM (1987) Prospective study on the dose relationship of mitomycin C induced interstitial pneumonitis. Cancer 60: 756
24. Wilson JKV, Byron HL, Marks ME, Brattain DE, Willey JE, Brattain MG (1984) Mitomycin C resistance in a human colon carcinoma cell line associated with cell surface protein alterations. Cancer Res 44: 5880