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MELPHALAN ANTITUMOR EFFICACY AND HEPATOTOXICITY

The effect of variable infusion duration in the hepatic artery

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RUNNING TITLE

Short-term infusion of melphalan in the hepatic artery

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ABSTRACT

The optimum conditions (duration, concentration) of a fixed dose, intra-arterial melphalan infusion in relation to its antitumor effect and toxicity in the liver were investigated in a rat colon tumor model (CC531) of liver metastases. We studied the difference in tumor and liver uptake, as well as antitumor effect and hepatotoxicity after 5 and 20-minute hepatic arterial infusion (HAI) of a fixed melphalan dose. Melphalan content in perfusate, liver and tumor tissue was analyzed by HPLC. The antitumor effect and hepatotoxicity in rats treated either systemically or with 5 and 20 min HAI with a fixed dose melphalan (4.4 μmol), were assessed two weeks after treatment. **N**o difference in melphalan content of tumor/liver tissue or antitumor effect was observed between rats treated with 5 and 20 min HAI. However, hepatotoxicity was strongly affected by perfusion duration/concentration: rats treated with 5 min HAI weighed significantly less and liver toxicity parameters were significantly increased compared to those of all other groups; 8 of 9 rats showed severe cholangiofibrosis. Body weights and liver toxicity parameters of the rats treated with 20 min HAI were not statistically different from the control group. In conclusion, duration of HAI with a 4.4 μmol fixed dose melphalan did not affect tumor uptake and antitumor effect, but the resulting increase in melphalan concentration had major impact on hepatobiliary toxicity. Therefore, in a clinical setting, caution should be taken when infusion duration and concentration of melphalan are increased.

Chemotherapeutic treatment is the therapy of choice when curative resection of liver metastases is not possible, for instance because too many metastases are present or the localization or size of these metastases precludes total resection. In the treatment of liver tumors hepatic artery infusion (HAI) of cytostatics is favored because liver tumors are mainly vascularized by the hepatic artery (HA) (Breedis C. and Young G., 1954; Sigurdson et al., 1987; Wang et al., 1994). Therefore, HAI of cytostatics leads to a more selective tumor exposure when compared to systemic administration (Sigurdson et al., 1987; Rothbarth et al., 2002a). Extensive preclinical and clinical research has been done on the antitumor effect and hepatotoxicity of HAI with 5-fluorouracil and fluorodeoxyuridine (Kemeny et al., 1992; Lorenz and Muller, 2000); currently HAI with these compounds is a well-established treatment modality for patients with colorectal liver metastases.

Isolated hepatic perfusion (IHP) is a relatively new local treatment for liver metastases and is only applied in a few centers in the world (Alexander, Jr. et al., 1998; Vahrmeijer et al., 2000): among others fluorouracil (Aigner et al., 1988) and melphalan (Alexander et al., 2000; Bartlett et al., 2001; Vahrmeijer et al., 2000) have been used as cytostatics. IHP involves a method of complete vascular isolation of the liver to allow treatment with drug doses that would cause severe toxicity when applied systemically. During IHP the liver is isolated from the systemic circulation for 1 hour to prevent systemic exposure to high doses of melphalan (Vahrmeijer et al., 2000; Rothbarth et al., 2002b).

Based on the advantage of HAI in terms of tumor exposure to cytostatics, melphalan is expected to be more effective if infused arterially during IHP. In a preclinical study in rats (Marinelli et al., 1991) and a phase I study in patients (Vahrmeijer et al., 2000) the maximum tolerated total doses of melphalan during IHP for both rats and patients have been determined. A study in rats confirmed the importance of arterial administration of melphalan (Rothbarth et al., 2002a). However, the optimum conditions (duration, concentration) of the intra-arterial melphalan infusion in relation to its antitumor effect and the safety are not known. For instance,

should the melphalan dose be infused over a short or long period of time during the vascular isolation of the liver? Obviously a short infusion time of the cytostatic compound in a clinical setting leads to a shorter duration of the whole procedure and therefore is preferable, but only when the antitumor effect is equal or better and the (hepato) toxicity is not increased.

When a fixed dose of melphalan is infused over a short period of time, the melphalan concentration of the infused volume is higher and the tumor is exposed to a higher melphalan concentration. Conversely, when the same melphalan dose is infused over a longer period of time, the tumor is exposed to a lower melphalan concentration, but for a longer period of time. We wanted to determine if there is a difference in tumor and liver uptake of melphalan, in antitumor effect and in hepatotoxicity between a short and long-term infusion as this could have consequences for the treatment strategy of our current clinical IHP program.

MATERIALS AND METHODS

Chemicals

Melphalan was purchased from Glaxo Wellcome Pharmaceuticals (Zeist, The Netherlands). A melphalan solution (16.4 mM) was prepared by dissolving 1 mg melphalan in 200 µl 0.09% (w/v) hydrochloric acid, which was subsequently diluted with Gelofusine®, a colloid solution of 4% gelatin in 0.9% NaCl, (Vifor Medical, Sempach, Switzerland) to a concentration of 480 µM; pH was adjusted to 7.4 with 1 N NaOH.

Rat model for colorectal liver metastases

The CC531 tumor cell line used is a carcinoma of the colon, syngeneic for WAG/Rij rats (Marquet et al., 1984). The cells were cultured in medium that consisted of RPMI 1640 medium supplemented with 10 % (v/v) fetal calf serum, 2 mM L-glutamine, 50 µg/ml streptomycin and 50 IU/ml penicillin (GIBCO, Life Technologies, Breda, the Netherlands). Cells were maintained by serial passage.

For tumor inoculation, exponentially growing cells were harvested by trypsinization, washed twice in phosphate buffered saline (PBS) and suspended at 1.0×10^7 tumor cells per ml. Male WAG/Rij rats (Harlan/CPB, Zeist, The Netherlands), anaesthetized using halothane, underwent laparotomy and tumor cells were inoculated at 3 or 4 sites by injecting 5×10^5 cells subcapsularly into the left and right main lobes and into the right accessory lobes of the liver.

Surgical procedures

The animals were anaesthetized by an intraperitoneal injection with a mixture of Hypnorm® (fentanyl citrate, 0.315 mg/ml; fluanisone, 10 mg/ml) (Janssen Pharmaceutics, Beerse, Belgium) and Dormicum® (midazolam) (Roche Nederland B.V., Mijdrecht, The Netherlands).

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(1) Melphalan concentration study: single pass liver perfusion

Rats bearing four liver tumors were used 12 days after tumor inoculation. At the time of IHP mean body weight was 289 ± 8 g (range 282-302). A single-pass isolated hepatic perfusion with melphalan infusion in the hepatic artery was performed in the normal flow direction as previously described (Pang and Terrell, 1981; Rothbarth et al., 2002a). In brief, after a V-line abdominal incision was made, the gastroduodenal artery and the pyloric vein were tied off. The common hepatic artery was cannulated with polyethylene tubing (PE-50, ∅ 0.61 mm). Perfusion of the liver was initiated upon cannulation of the portal vein with a 16G double-needle cannula. The inferior caval vein above the kidneys was tied to ensure unidirectional flow, whereas the lower abdominal inferior caval vein close to the extremities was severed to allow immediate drainage. The diaphragm was then opened and a 16G cannula was placed in the suprahepatic inferior caval vein through the right atrium to collect the outflow from the hepatic veins. The liver was perfused through the hepatic artery and portal vein using the caval vein for the outflow. The perfusion circuit consisted of a low-flow roller pump (Watson-Marlow BV, Rotterdam, The Netherlands), an infusion pump (perfusor, B. Braun, Melsungen, Germany), a collection reservoir/oxygenator and a heat exchanger.

The perfusate, consisting of Gelofusine® with 20% (v/v) outdated washed human erythrocytes (courtesy of the Blood Bank Leiden, The Netherlands), was oxygenated (95% oxygen; 5% carbon dioxide) and kept at 37 ^{o}C. The pH was adjusted to 7.4 with sodium hydrogen carbonate 8.4%. The liver was perfused through the portal vein at a flow rate of 10 ml/min and through the hepatic artery at a flow rate of 1.0 ml/min; the melphalan solution was added to the hepatic artery perfusate by an infusion pump at a flow rate of 0.5 ml/min. At the end of the perfusion a wash out was performed through the portal vein with 30 ml of ice-cold saline during 3 minutes.

A total amount of 0.9 mg (2.9 μmol) melphalan was infused in the hepatic artery in either 5 or 20 minutes. When the arterial infusion of melphalan was started, samples of the effluent of

the caval vein were taken at 0, 1, 2, 3, 4, 5, 10, 15 and 20 minutes. After the liver was excised, the tumors were removed from the liver. All samples were stored at -70 ^{o}C until analysis.

(2) Melphalan antitumor effect and toxicity study in the rat in vivo

Rats bearing three liver tumors were treated with HAI at 10 days after tumor cell inoculation. At time of HAI treatment mean body weight was 234 ± 10 g (range 215-255) and the mean cross-sectional tumor area was 26.3 mm^2. For hepatic artery infusion a cannula (PE-50, ∅ 0.61 mm) was inserted into the gastroduodenal artery with the tip in the common hepatic artery leaving normal arterial blood flow intact. After infusion the gastroduodenal artery was tied off. For systemic infusion a cannula (PE-50, ∅ 0.61 mm) was inserted in a lumbal vein, which was tied off after infusion.

The rats were randomly assigned to the following treatment groups: (1) 25 min saline via HAI (control group), (2) 10 min systemically infused melphalan and 25 min saline via HAI (systemic melphalan group), (3) 5 min melphalan followed by 20 min saline via HAI (5 min HAI melphalan group) and (4) 20 min melphalan followed by 5 min saline via HAI (20 min HAI group). All melphalan treated rats received a total dose of 1.35 mg (4.4 μmol) melphalan infused over a period of 5, 10 or 20 min. HAI was performed with an infusion pump (perfusor, B. Braun, Melsungen, Germany) at a flow rate of 25 μl/min. Before treatment the cross-sectional tumor area was determined (estimated by caliper measurements and calculated as: π x 0.25 x largest diameter x pendicular diameter).

Toxicity was assessed by effects on survival, body weight (4 days, 1 week and 2 weeks after treatment), serum levels of sodium (Na), potassium (K), creatinine, bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (AP) (blood samples at day of tumor cell inoculation and 1 week and 2 weeks after treatment).

To determine the antitumor effect of the different treatments, rats were sacrificed two weeks after treatment, the tumors were weighed and the tumor growth index was calculated. The

tumor growth index was defined as cross-sectional tumor area two weeks after treatment divided by the cross-sectional tumor area at day of treatment.

After the rats were sacrificed the livers were examined macroscopically and microscopically. For histological examination 5 μm thick cryo sections were cut from snap frozen liver tissue. Sections were dried overnight at 60 ^{o}C and fixed in acetone. Sections were stained with haematoxylin and eosin (H&E).

Melphalan concentration assay

The concentration of melphalan in the effluent samples as well as in tumor and liver tissue samples was measured using high-performance liquid chromatographic (HPLC) assay as previously described (Rothbarth et al., 2002a).

Statistical analysis

All data were analyzed with SPSS statistical software (version 9.0 for Windows; SPSS, Chicago, IL). Correlation coefficients were calculated using the paired student t-test. A p value < 0.05 was considered statistically significant.

RESULTS

Melphalan concentration study: single pass liver perfusion

We investigated tumor and liver uptake of melphalan in a single-pass IHP after 5 and 20 minutes arterial infusion of a fixed dose of melphalan: tumor-bearing rat livers were perfused for either 5 or 20 minutes with a total dose of 0.9 mg (2.9 μmol) melphalan in the hepatic artery in each case. The melphalan dose was based on the dose currently used in the clinical IHP: 3.0 mg kg^{-1} (Vahrmeijer et al., 2000).

HPLC analysis of tumor and liver tissue revealed that uptake in both tumor and liver of arterially infused melphalan was indeed very similar in the two groups (Fig. 1). There was no statistical difference in the tumor and liver content of melphalan after either 5 or 20 minutes single-pass IHP. As expected (Rothbarth et al., 2002a) melphalan concentration in tumor tissue was much higher than in liver tissue in both groups ($p < 0.05$). A rapid extraction of melphalan by the liver and tumor occurred during the first 5 minutes in both groups (Fig. 2); in the 20 min HAI group steady state was reached after 10 minutes. In concordance with the arterial inflow concentrations in the 5 min and 20 min HAI groups (0.12 mg ml^{-1} and 0.03 mg ml^{-1} respectively), the concentration of melphalan in the effluent of the 5 min HAI was approximately 4 times higher than that of the 20 min HAI group (Fig. 2).

Melphalan antitumor effect and toxicity study in the rat in vivo

For the study on the antitumor effect and toxicity after different modes of melphalan administration, 9 rats were treated with 5 min HAI with melphalan; in the control, systemic melphalan and 20 min HAI melphalan groups 8 rats were treated.

Four of the 9 rats in the 5 min HAI melphalan group died or were sacrificed because of bad physical condition before the end of the experiment (two at day 6, one at day 8 and one at day 10 after treatment). The antitumor effect could not be evaluated in one surviving rat because necrotic cavities filled with pus were present at the tumor sites; therefore, the volume of tumor

tissue could not be assessed. As a result the antitumor effect after treatment could only be evaluated in 4 out of 9 rats in the 5 min HAI melphalan group. In the other three groups none of the rats died prematurely and antitumor effect after treatment was evaluable in all rats.

There was no statistically significant difference between the antitumor effect of a 5 and 20 min HAI with melphalan as determined by both the tumor growth index and the average tumor weight at the end of the experiment (Table 1). The 5 and 20 min HAI melphalan groups showed a strongly decreased tumor weight and tumor growth index as compared to the control group ($p < 0.05$) The antitumor effect was 2 times higher when compared to the group that received systemically administered melphalan ($p < 0.05$ for 20 min HAI melphalan group, $p = 0.18$ for the 5 min HAI melphalan group) as determined by the tumor growth index. The average tumor weights of the 5 and 20 min HAI melphalan groups were 6 to 10 times lower than of the control group ($p < 0.05$) and 2 to 3 times lower than of systemically administered melphalan group ($p < 0.05$ for 20 min HAI melphalan group, $p = 0.11$ for the 5 min HAI melphalan group).

Rat body weight was strongly affected by the type of treatment (Fig. 3). Rats treated with 5 min HAI with melphalan weighed significantly less than rats of all other groups at 4 days, 1 week and 2 weeks after treatment ($p < 0.05$). As mentioned before, 4 rats of this group (5 min HAI) died before the end of the experiment; at the time of death mean body weight was 171 ± 7 g (range 161-176). Rats treated with 20 min HAI or with systemically administered melphalan initially weighed less than those of the control group at 4 days ($p < 0.05$ both groups) and 1 week ($p < 0.05$ systemic melphalan group only) after treatment (Fig. 3). Two weeks after treatment, however, there was no statistical difference between the body weights of the rats treated with systemic and 20 min HAI melphalan on one hand and the control group on the other.

In the control, systemic melphalan and 20 min HAI melphalan groups no changes in serum levels of sodium, potassium, creatinine and liver toxicity parameters (ASAT, ALAT, AP and bilirubin) were found 1 and 2 weeks after treatment. Liver toxicity parameters of the 5 min HAI

melphalan group were significantly higher compared to those of all other groups both 1 and 2 weeks after treatment ($p < 0.05$) (Table 2).

Macroscopic examination of the rat livers showed clear signs of cholestasis in 8 of the 9 rats treated with 5 min HAI and 1 of the rats treated with 20 min HAI with melphalan: massive bile accumulation in the liver, yellow-spotted livers, yellow-brown skin and sclera and swelling and obstruction of the bile duct. In addition, sera of these rats were yellow colored. All rats showing macroscopic signs of cholestasis had elevated bilirubin levels 1 week (mean 42 μmol/L, range 6-76) and 2 weeks (mean 97 μmol/L, range 32-168) after treatment. Microscopic examination of these rat livers showed atypical glandular structures lined by basophilic, occasionally dysplastic epithelium ranging from flattened to cuboidal cells. The presence of connective tissue in all of these rat livers indicated cholangiofibrosis (Goodman et al., 1994) (Fig. 4). None of the other rats showed macroscopic signs of cholestasis or histological signs of cholangiofibrosis.

DISCUSSION

Local administration of cytostatics in the hepatic artery leads to more selective liver tumor exposure (Sigurdson et al., 1987; Rothbarth et al., 2002a) and consequently to safer tumor treatment when compared to systemic administration of cytostatics, including higher tumor response rates. This has been confirmed by many clinical studies (Chang et al., 1987; Hohn et al., 1989; Kemeny et al., 1987; Vahrmeijer et al., 1995; Kemeny, 1995; Meta-Analysis Group in Cancer, 1996; Lorenz et al., 2001). However, no information is available on the optimum infusion duration. A short infusion time of the cytostatic compound in a clinical setting obviously leads to a shorter duration of the whole procedure and therefore is preferable. This study was performed to find out how infusion duration of melphalan affects tumor melphalan uptake and anti-tumor efficacy on one the one hand and liver toxicity on the other.

Our results show that there is no difference in tumor and liver uptake of melphalan after short-term (5 minutes) or long-term (20 minutes) arterial infusion of a fixed dose of melphalan. Evidently, when the total administered amount of melphalan (based on a clinically relevant dose) is equal, neither duration of melphalan exposure nor melphalan concentration affect the tumor and liver uptake of melphalan within the conditions of our experiment. Based on these results, no differences in antitumor effect would be expected after 5 or 20 min arterial infusion either: melphalan exerts its cytotoxic effect by formation of DNA-adducts (Kohn, 1981) and a clear correlation has been observed between melphalan concentration, level of melphalan derived DNA-adducts and (Tilby et al., 1993) cytotoxicity (Frank et al., 1996; Tilby et al., 1993; Hansson et al., 1987).

Indeed there was no significant difference in antitumor effect between 5 and 20 min arterial infusion of the fixed dose of melphalan ($p < 0.05$). To exclude the possibility that this equal antitumor effect was simply due to the fact that all rats in these two groups received an equal amount of melphalan and, therefore, had an equal total body exposure of melphalan, another group of rats was treated systemically with the same amount of melphalan. In this group the

antitumor effect was 2 to 3 times less when compared to the HAI groups. Therefore, the additional antitumor effect of melphalan in both the 5 and 20 min HAI group must be due to the arterial administration of melphalan; this confirmed the route-dependent effect of melphalan administration. Thus, the antitumor effect of 5 min and 20 min HAI is equal and, as a shorter infusion time is desirable, 5 min HAI would be preferred.

Unfortunately, the group of rats treated with 5 min HAI with melphalan experienced major hepatobiliary toxicity. Histochemical examination showed cholangiofibrosis in the liver of our rats, which histopathologically is similar to biliary sclerosis in humans, a well known complication of arterially infused fluorodeoxyuridine (Hohn et al., 1985; Kemeny et al., 1992; Rougier et al., 1992; Lorenz and Muller, 2000).

The fact that mainly biliary toxicity is seen in hepatic arterial infusion therapy might be explained by the difference in arterial and portal blood supply between bile ducts and liver parenchyma. The liver parenchyma is perfused by the hepatic artery and portal vein at physiologic ratio of arterial to portal flow of about 1:3 (Rappaport, 1980; Watanabe et al., 1994; Watanabe et al., 1994). Bile ducts, however, are, mainly vascularized by the hepatic artery and not the portal vein (Cho and Lunderquist, 1983; Mitra, 1966; Northover and Terblanche, 1979), just like liver tumors (Breedis C. and Young G., 1954; Sigurdson et al., 1987; Wang et al., 1994). Consequently, the bile duct is exposed to a much higher drug concentration compared to the liver parenchyma when infused in the hepatic artery. Liver parenchyma is not exposed to the high arterial concentration, because arterially infused drugs are substantially diluted by portal venous blood. This may be the explanation for the fact that mainly hepatobiliary toxic effects occur after hepatic arterial drug infusion, as is the case in our experiments.

In the current experiments the tumor response was not affected by melphalan *concentration* as long as the tumors were exposed to the same *amount* of melphalan. Unfortunately, the concentration-toxicity curve appeared to be very steep, indicating that once the toxicity

threshold concentration is reached, a small increase in melphalan concentration leads to a large increase in toxicity.

How would these results "translate" to the clinical situation? Assuming that hepatic arterial flow in the our rats was between 1 and 3 ml min^{-1} (≈ 4.3 to 12.8 ml min^{-1} kg^{-1}) (Daemen et al., 1989; Tanaka et al., 1999), the calculated arterial melphalan concentration was 0.02–0.05 mg ml^{-1} for the 20 min HAI and 0.07–0.20 mg ml^{-1} for the 5 min HAI group. In our current clinical IHP, a total of 200 mg melphalan is infused over 20 minutes in the hepatic artery at a flow rate of 100 ml min^{-1}, resulting in a concentration of 0.1 mg ml^{-1}. As this is already within the concentration range of the 5 min HAI group and as most patients already experience a transient elevation of liver toxicity parameters (unpublished data), a further increase in hepatic arterial infusion concentration is not favored. Consequently, caution should be taken when the infusion concentration of melphalan of such a treatment regimen is increased.

In conclusion, the current results reveal that the duration of HAI with a fixed dose melphalan does not affect the tumor uptake of melphalan and the antitumor effect, but that relatively small changes in melphalan concentration in the hepatic artery can have major impact on hepatobiliary toxicity.

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FOOTNOTES

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TABLES

Table 1. Antitumor effect on rat liver tumors after different modes of melphalan administration

	Evaluable rats (n)	Tumor growth index	Tumor weight (mg)
Control	8	4.0 ± 0.7	0.79 ± 0.46
Systemic melphalan	8	1.9 ± 1.4*	0.26 ± 0.08*
5 min HAI melphalan	4†	0.8 ± 0.6*	0.12 ± 0.10*
20 min HAI melphalan	8	0.7 ± 0.4**	0.08 ± 0.05**

Tumor growth index and tumor weight two weeks after treatment. The tumor growth index was defined as cross-sectional tumor area two weeks after treatment divided by the cross-sectional tumor area at day of melphalan treatment. Values are mean ± SD. *Statistically significant different from control group, **statistically significant from control and systemic melphalan group ($p < 0.05$). †Four rats died before evaluation was possible and in 1 rat the presence of tumor tissue could not be exactly identified (see Fig. 3 and Results section).

Table 2. Effect of the different treatments on blood serum levels

	Control		Systemic melphalan		5 min HAI melphalan		20 min HAI melphalan	
	Post-treatment levels		Post-treatment levels		Post-treatment levels		Post-treatment levels	
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
	(*n* = 8)	(*n* = 8)	(*n* = 8)	(*n* = 8)	(*n* = 7)†	(n = 5)†	(*n* = 8)	(*n* = 8)
Na (mmol/L)	142 (2)	142 (2)	142 (3)	141 (2)	141 (2)	141 (6)	144 (2)	141 (2)
K (mmol/L)	4.9 (0.5)	5.7 (0.8)	4.7 (0.7)	7.0 (1.2)	4.6 (0.6)	5.7 (1.5)	5.0 (0.3)	6.7 (1.2)
Creatinine (μmol/L)	39 (2)	36 (9)	38 (5)	33 (4)	38 (10)	36 (8)	39 (3)	36 (4)
Bilirubin (μmol/L)	1.5 (0.6)	1.5 (0.5)	1.0 (0.5)	2.0 (0.5)	36 (26)*	71 (59)*	2.0 (23.8)	2.0 (51.4)
ASAT (U/L)	132 (81)	218 (89)	96 (16.9)	135 (49)	1366 (4052)*	1376 (2727)*	103 (175)	131 (248)
ALAT (U/L)	78 (7)	83 (25)	61 (27)	87 (11)	229 (280)*	294 (259)*	70 (35)	82 (51)
AP (U/L)	185 (28)	131 (12)	145 (42)	181 (41)	924 (690)*	909 (1386)*	178 (95)	164 (91)

Levels of sodium (Na), potassium (K), creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP) levels 1 and 2 weeks after melphalan treatment. Values are median (SD). *Statistically significant difference from all other groups at the same time point ($p < 0.05$). †Two rats died within 1 week and another 2 rats within 2 weeks after melphalan treatment.

FIGURE LEGENDS

Fig. 1. Mean concentration of melphalan in tumor and liver tissue (±SD) after single-pass isolated hepatic perfusion with arterial administration of a fixed dose melphalan in 5 or 20 min (n = 5).

Fig. 2. Melphalan concentration in the outflowing perfusate (±SD) after single-pass isolated hepatic perfusion with arterial administration of a fixed dose melphalan in 5 (■) or 20 minutes (□) (n = 5).

Fig. 3. Average change in weight of male Wag/Rij rats after different treatments: ■ control (n = 8), ● systemic melphalan (n = 8), □ 20 min HAI melphalan (n = 8) and ○ 5 min HAI melphalan (n = 9). Four rats in the 5 min HAI melphalan group died before the end of the experiment (+); the number of rats evaluated per time point is indicated between brackets. Mean body weight on day 0 was 234 ± 10 gram (range 215-255). *Significantly different compared to weights after all other treatments. #Significantly different compared to weights of control group ($p < 0.05$).

Fig. 4. Histological section of a control rat liver (a) and a rat liver treated with 5 min HAI with melphalan (b) (H&E, 200x). The control rat liver (a) shows a normal bile duct (thin arrow). The rat liver treated with 5 min HAI with melphalan shows severe cholangiofibrosis (b). This lesion is characterized by atypical glandular structures linee by basophilic, occasionally dysplastic epithelium ranging from flattened to cuboidal cells (thin arrow). Connective tissue is clearly present. Multifocal areas of cholangiofibrosis often are continuous with one another as is also the case in this figure (thick arrow).

Figure 1

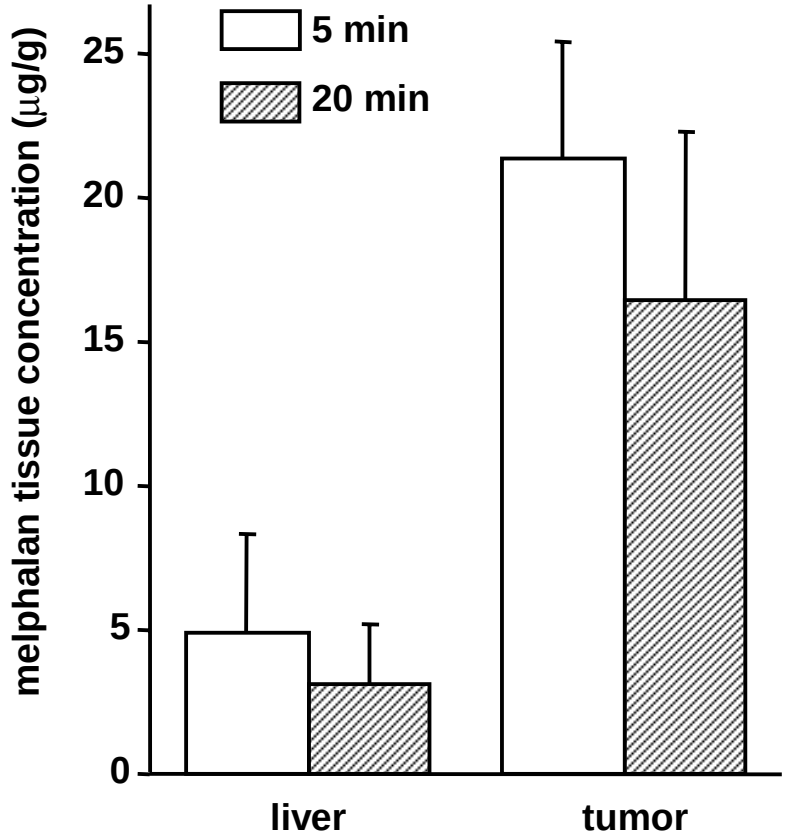


Figure 2

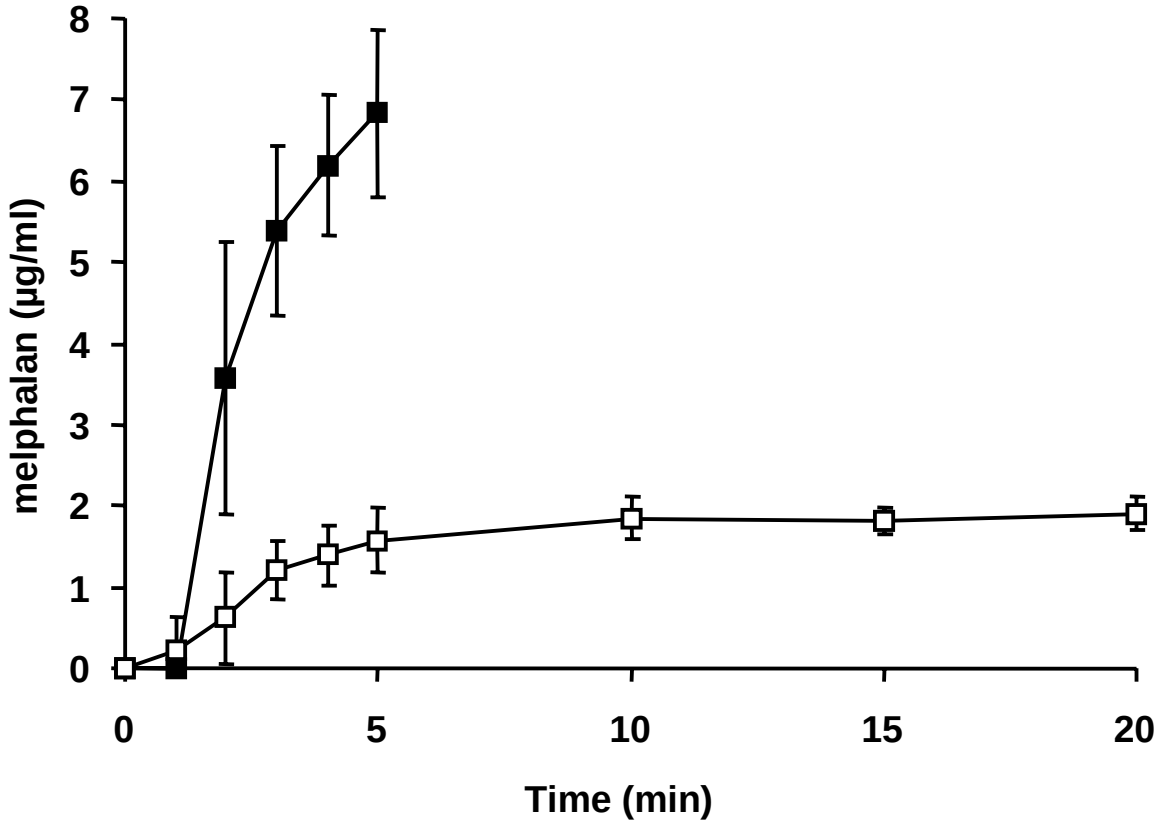
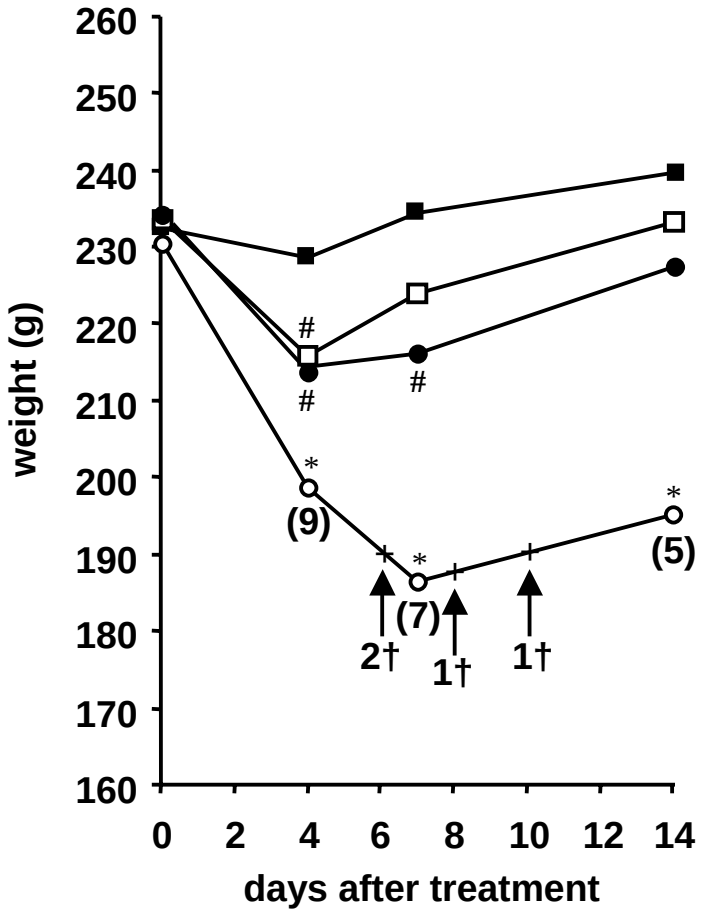


Figure 3



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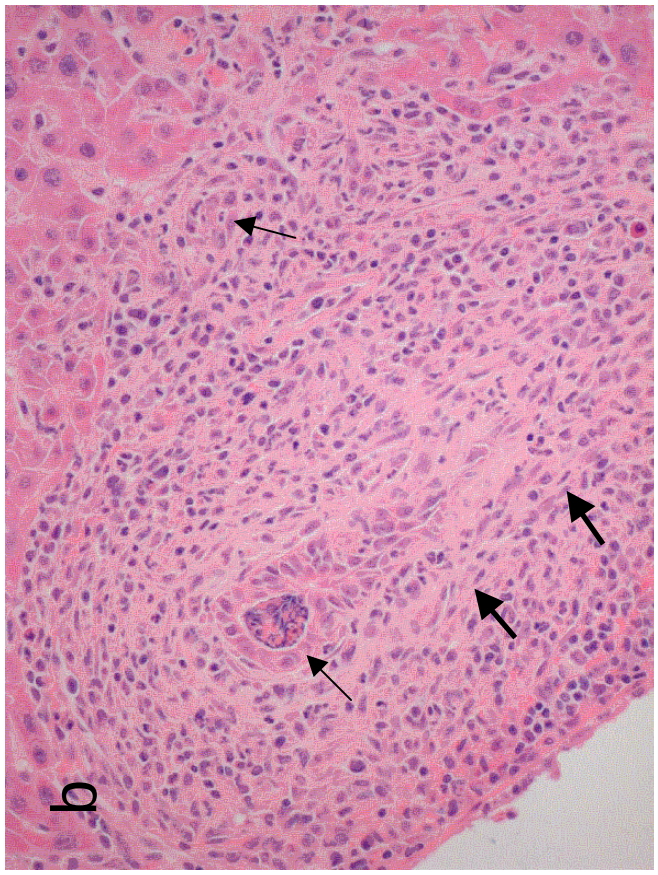

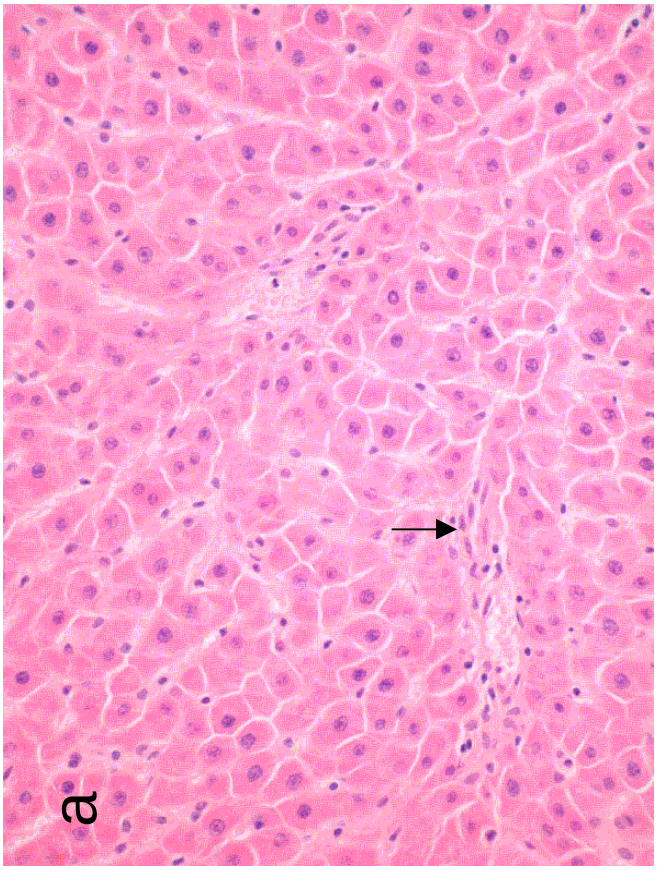

Figure 4