Hepatic Arterial Infusion with Tumor Necrosis Factor- α Induces Early Hepatic Hyperperfusion

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Key Words

Hepatic arterial infusion · Tumor necrosis factor- α ·
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Abstract

Background: Hepatic arterial infusion (HAI) has been developed for high-dose regional chemotherapy of unresectable liver metastases or primary liver malignancies. While it is well known that high concentrations of tumor necrosis factor (TNF)- α damage tumor blood perfusion, there is no information on whether autochthonous liver perfusion is affected by HAI with TNF- α . Therefore, we investigated the effects of HAI with TNF- α on hepatic macro- and microvascular perfusion.

Methods: Swabian Hall pigs were randomized into three groups. HAI was performed with either 20 or 40 $\mu\text{g/kg}$ body weight TNF- α ($n = 6$ each group). Saline-treated animals served as controls ($n = 6$). Analyses during a 2-hour post-HAI observation period included systemic hemodynamics, portal venous and hepatic arterial blood flow, portal venous pressure, and the blood flow in the hepatic microcirculation.

Results: HAI with TNF- α caused a slight decrease of mean arterial blood pressure ($p < 0.001$), which was compensated by a moderate increase of heart rate ($p < 0.001$). No further systemic side effects of TNF- α were observed. HAI with TNF- α further caused a slight but not significant decrease of por-

tal venous blood flow ($p = 0.737$) in both experimental groups, paralleled by an increase of hepatic arterial blood flow ($p = 0.023$, 20 $\mu\text{g/kg}$; $p = 0.034$, 40 $\mu\text{g/kg}$) resulting in an overall hepatic hyperperfusion. The hepatic hyperperfusion after HAI with 20 $\mu\text{g/kg}$ TNF- α was more pronounced and associated with a 40% decrease of the blood flow in the hepatic microcirculation ($p = 0.009$). HAI with 40 $\mu\text{g/kg}$ TNF- α was only associated with a temporary and moderate total hepatic hyperperfusion and did not affect the blood flow in the hepatic microcirculation. **Conclusion:** HAI with TNF- α causes a decrease of portal venous flow; however, this is overcompensated by an increased hepatic arterial blood flow, resulting in a total hepatic hyperperfusion. Moderate total hepatic hyperperfusion does not affect the blood flow in the hepatic microcirculation, while a persistent and more pronounced hyperperfusion may cause hepatic microcirculatory disturbances.

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Introduction

It is well known that blood supply of liver metastases is mainly derived from the hepatic artery (HA), whereas the normal liver tissue is supplied by both the portal venous (PV) and the hepatic arterial system [1, 2]. Based on

this knowledge, hepatic arterial infusion (HAI) has been developed for regional chemotherapy of liver metastases [3]. Due to the first pass effect of the liver and the predominant arterial blood supply of tumors, HAI results in higher exposure of the tumor tissue to chemotherapeutic drugs and less systemic side effects than systemic application [4, 5]. Although several clinical studies have reported increased response rates of metastases after HAI [6–10], a survival benefit has only been proven in few studies [7]. Thus, HAI has not yet become a standard therapy for the treatment of liver metastases [11].

However, recent studies with modern anticancer drugs eventually may redefine the potentials of HAI for eradication of liver metastases [12–14]. Substantial benefit in the treatment of unresectable hepatic tumors has been shown by combining high-dose regional chemotherapy, predominantly melphalan [15–18], with the application of tumor necrosis factor (TNF)- α . Because of its serious systemic side effects, including vasoplegia, TNF- α has only been given in isolated hepatic perfusion [15] and isolated limb perfusion [19]. Recombinant human TNF- α has been demonstrated to exert an inhibiting effect on angiogenic tumor vessels [20] and, additionally, to induce distinct damaging effects on the tumor-supplying vasculature [21]. The latter include the increase of the endothelial permeability [22], leading to improved chemotherapy penetration to the tumor tissue [23], and a selective destruction of angiogenic endothelial cells, resulting in tumor vessel obliteration with breakdown of the nutritional tumor perfusion and extensive tumor necrosis [24, 25].

Apart from these direct effects of TNF- α on the tumor vasculature, little is known about the effects of TNF- α on the host vasculature. After application of TNF- α via the HA, the highest TNF- α concentration should be found in the arterial branches of the hepatic microcirculation. So far, however, it is not known to which extent autochthonous liver perfusion is affected by application of TNF- α in HAI. As there is no data for nonisolated liver perfusion with TNF- α available thus far, choosing the dosage has to be oncologically effective without being harmful to the patient. Therefore, TNF- α doses of both 20 and 40 $\mu\text{g/kg}$ body weight were chosen, as such doses have been shown to be effective in isolated liver perfusion and isolated limb perfusion. In an experimental porcine model, the effects of HA infusion of these different doses of TNF- α on hepatic circulation and microvascular perfusion were analyzed.

Material and Methods

The experiments were approved by the local ethics committee (Landesamt für Gesundheit und Verbraucherschutz, ethical approval code: 32/04) and conformed to the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia and the NIH Guide for Care and Use of Laboratory Animals.

Animals and Anesthesia

Eighteen pigs of the Swabian Hall strain of either sex with a mean body weight of 24.3 ± 1.2 kg ($p = 0.899$) were used. Animals had free access to tap water and a standard diet (Raiffeisen, Cologne, Germany). After a 7-day adaptation period, food, but not water, was withheld for 24 h before the experiments. After intramuscular premedication with 10 mg/kg azaperone (Stresnil; Janssen, Neuss, Germany) and 10 mg/kg metomidate hydrochloride (Hypnodil; Janssen), general anesthesia was induced by intravenous injection of 1 mg/kg etomidate (Hypnomidate; Janssen) into an ear vein. The animals were then intubated and ventilated mechanically (Dräger Avl; Dräger, Lübeck, Germany) with a mixture of oxygen and room air (FiO_2 0.30, PEEP 5 mm Hg). The ventilation was adjusted to maintain the PaO_2 above 13.3 kPa and the PaCO_2 between 4.7 and 5.3 kPa at baseline. Balanced anesthesia was achieved throughout the experiment by intravenous infusion of 2–4 mg/kg/h thiopental sodium (Trapanal; Byk Gulden, Konstanz, Germany) through a central venous catheter inserted into the right internal jugular vein. NaCl (0.9%; 154 mmol/l) was infused intravenously at a rate of 12–15 ml/kg/h throughout the experiment [26]. At the end of the experiment, the animals were killed by intravenous injection of a lethal dose of thiopental sodium while the animals were fully anesthetized.

Surgical Preparation and Hemodynamic Analysis

The anesthetized animals were placed in a supine position on a warming blanket. Continuous fluid supply was performed via a central venous catheter. Mean arterial blood pressure (MAP) was continuously monitored by connection of a catheter in the left femoral artery to a pressure gauge (Statham Typ P23 ID; Gould Inc., Oxnard, Calif., USA).

After midline laparotomy, the splenic vein was cannulated to measure the portal venous pressure (PVP) and the gastroduodenal artery was cannulated to perform the HAI.

Flow Measurement

To measure hepatic blood perfusion, the hepatoduodenal ligament was dissected and precalibrated ultrasonic transit-time flow probes (Transonic Systems Inc., Ithaca, N.Y., USA) were placed around the HA and the portal vein. The flow probes were connected to an ultrasound blood flow meter (T206 Animal Research Flowmeter, Transonic Systems) [27–29]. This allowed the simultaneous assessment of HA and PV blood flow without the risk of mechanical obstruction or kinking of the blood vessels [27].

Laser Doppler Fluxmetry

For monitoring of hepatic microvascular blood flow, laser Doppler fluxmetry (LDF; PeriFlux System 5000, Perimed, Stockholm, Sweden) was used [30, 31]. Blood flow was measured by placing the laser Doppler probe on three different areas of the

liver surface in the center of the right and the left lobe. Each measurement was recorded for at least 30 s. Care was taken to ensure continuous and steady contact with the tissue under investigation and to prevent motion disturbances from respiration and gastrointestinal peristalsis throughout the experiment [32].

Experimental Protocol

At the end of surgical preparation, 30 min were allowed for stabilization before at least 5 baseline readings were obtained. To study the effects of TNF- α on the hepatic perfusion, animals were randomly allocated to three groups. In the first group, HAI was performed with normal saline infusion (0.9% NaCl; $n = 6$). In group 2, HAI was performed with 20 $\mu\text{g}/\text{kg}$ body weight TNF- α (recombinant human TNF- α ; $4.9\text{--}5.5 \times 10^7$ U/mg; Boehringer Ingelheim GmbH, Ingelheim, Germany; $n = 6$). In group 3, HAI was performed with 40 $\mu\text{g}/\text{kg}$ body weight TNF- α ($n = 6$). HAI was performed over a 15-min period with an infusion rate of 100 ml/h by means of an electronically steered syringe pump (B. Braun, Melsungen, Germany), which was filled with 25 ml physiological saline and the body weight-adapted amount of TNF- α . During HAI, no additional fluid was given. After HAI, systemic and hepatic hemodynamic parameters were recorded every 10 min for a total of 2 h. Thereafter, 250 ml HAES (6%) was administered for resuscitation [33] during 10 min in all groups and hemodynamic parameters were recorded for a further 30 min.

Statistics

All values are expressed as means $\pm$ SEM. After analysis of normal distribution and equal variance of the data, differences within each group were calculated by a one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test, which included the correction of the α -error according to Bonferroni probabilities to compensate for multiple comparisons. Differences between the three groups were analyzed by ANOVA followed by the Student-Newman-Keul test. Overall statistical significance was set at $p < 0.05$. Statistical analysis was performed with the use of SigmaStat (SPSS Inc., Chicago, Ill., USA).

Results

All animals survived the surgical preparation as well as the HA infusion with 20 or 40 $\mu\text{g}/\text{kg}$ TNF- α . During the entire observation period no complications like bleeding, cardiac failure, pulmonary dysfunction, or lung edema could be observed. Body temperature at baseline was $35.7 \pm 0.3^\circ\text{C}$ ($p = 0.681$) and declined during the observation period to $34.3 \pm 0.4^\circ\text{C}$ ($p = 0.377$) without any significant differences between the groups. At the beginning of the experiments, hematocrit was $24.9 \pm 0.8\%$ ($p = 0.867$) and decreased slightly to $23.7 \pm 0.9\%$ ($p = 0.777$) due to repeated blood withdrawals and surgery-associated blood loss. However, there was no significant difference between the baseline hematocrit and the hematocrit at the end of the experiment.

Fig. 1. MAP (a) and HR (b) in percent of baseline (base) and during HAI as well as during the 120-min post-HAI period and during the 30 min after HAES resuscitation (RES). HAI was performed either with NaCl ($\circ$) or 20 $\mu\text{g}/\text{kg}$ TNF- α ($\bullet$) and 40 $\mu\text{g}/\text{kg}$ TNF- α ($\blacktriangle$). Mean $\pm$ SEM; * $p < 0.05$ vs. NaCl controls.

Systemic Hemodynamics

At baseline, MAP in control animals was 115 ± 5 mm Hg. During HAI and after the first 15 min following HAI, MAP decreased about 18%, but spontaneously normalized during the subsequent 2-hour observation period (fig. 1a). MAP was not significantly affected by resuscitation with 250 ml HAES. HAI with 20 $\mu\text{g}/\text{kg}$ TNF- α (baseline 121 ± 6 mm Hg) and in particular with 40 $\mu\text{g}/\text{kg}$ TNF- α (baseline 113 ± 10 mm Hg) resulted in a 20 and 30% decrease of MAP ($p < 0.001$; fig. 1a). Resuscitation with 250 ml HAES effectively restored MAP in TNF- α -treated animals to values almost similar to that of controls (fig. 1a).

In control animals, heart rate (HR) at baseline was $77 \pm 3 \text{ min}^{-1}$. In line with the decrease of MAP, HR in controls slightly decreased during HAI and the following 15

Fig. 2. PV flow (a), hepatic arterial flow (HA flow; b) and THBF (c) at baseline (base) and during HAI as well as during the 120-min post HAI period and during the 30 min after HAES resuscitation (RES). HAI was performed either with NaCl (○) or 20 µg/kg TNF-α (●) and 40 µg/kg TNF-α (▲). Mean ± SEM; * $p < 0.05$ vs. NaCl controls.

min, but spontaneously normalized to baseline level. Resuscitation with HAES did not influence HR (fig. 1b). TNF-α treatment in a dose of 20 µg/kg caused an approximate 25% increase of HR at 1 h after HAI ($p < 0.001$) which spontaneously normalized by 120 min of observa-

tion. Resuscitation with HAES did not significantly affect HR in these animals (fig. 1b). In animals treated with 40 µg/kg TNF-α, HR was found increased over the entire 120-min observation period ($p < 0.001$). Of interest, resuscitation with HAES immediately normalized HR to baseline levels (fig. 1b).

PV and Hepatic Arterial Blood Flow

In control animals, PV blood flow at baseline was 687 ± 76 ml/min/kg liver weight. HAI with saline caused a decrease of PV blood flow to 563 ± 35 ml/min/kg liver weight, from 762 ± 69 to 552 ± 50 ml/min/kg liver weight (20 µg/kg), and from 675 ± 90 to 531 ± 87 ml/min/kg liver weight (40 µg/kg), respectively, which persisted over the entire 120-min observation period. Resuscitation with HAES restored PV blood flow (fig. 2a). HAI with 20 µg/kg TNF-α decreased PV blood flow by approximately 25% during HAI and the following 10 min. This depression of PV blood flow spontaneously recovered, presenting with baseline values significantly exceeding PV blood flow values of controls ($p = 0.048$). Resuscitation with HAES caused a significant increase of PV blood flow ($p = 0.046$; fig. 2a). Of interest, HAI with 40 µg/kg TNF-α caused a less pronounced decrease of PV blood flow compared to that observed after HAI with 20 µg/kg TNF-α, which, however, was persistent over the entire 120-min observation period. Resuscitation with HAES restored PV blood flow (fig. 2a).

In control animals hepatic arterial blood flow was 274 ± 51 ml/min/kg liver weight at baseline and remained unaffected during the entire course of the experiment. HAI with 20 µg/kg TNF-α caused a 2.5-fold increase of HA blood flow from 278 ± 54 to 660 ± 133 ml/min/kg liver weight within the first 15 min after TNF-α application ($p = 0.023$; fig. 2b). Of interest, HA blood flow remained elevated throughout the entire observation period. In animals treated with 40 µg/kg TNF-α, HA blood flow also showed an early increase after TNF-α application from 299 ± 51 ml/min/kg liver weight ($p = 0.034$; fig. 2b), although the peak value of 547 ± 106 ml/min/kg liver weight was lower compared to that measured after application of 20 µg/kg TNF-α. In both groups, resuscitation did not affect HA blood flow (fig. 2b).

In control animals, total hepatic blood flow (THBF) was 961 ± 111 ml/min/kg liver weight at baseline. According to PV and HA blood flow, THBF remained unchanged during the entire course of the experiment. In animals treated with 20 µg/kg TNF-α, THBF was found significantly increased (from $1,039 \pm 111$ to $1,359 \pm 149$ ml/min/kg liver weight) after HAI ($p = 0.019$; fig. 2c). In ani-

Fig. 3. PVP in percent of baseline (base) and during HAI as well as during the 120-min post-HAI period and during the 30 min after HAES resuscitation (RES). HAI was performed either with NaCl (○) or 20 µg/kg TNF-α (●) and 40 µg/kg TNF-α (▲). Mean $\pm$ SEM; * $p < 0.05$ vs. NaCl controls.

mals treated with 40 µg/kg TNF-α, THBF was also found elevated after HAI (from 974 ± 94 to $1,187 \pm 196$ ml/min/kg liver weight; $p = 0.041$), although this increase was less pronounced compared to that observed after application of 20 µg/kg TNF-α. In both TNF-α-treated groups, resuscitation caused a further increase of THBF (fig. 2c).

Portal Venous Pressure

In control animals PVP was 3.6 ± 0.6 mm Hg at the beginning of the experiments. Upon HAI with saline, PVP decreased slightly, but recovered over the course of the experiment. Resuscitation induced an approximate 50% increase of PVP within the first 10 min after HAES application (fig. 3). Treatment with 20 µg/kg TNF-α provoked a temporary increase of PVP already during HAI (from 6.3 ± 0.3 to 8.3 ± 0.7 mm Hg; $p = 0.008$; fig. 3), which normalized during the subsequent observation period. In contrast, HAI with 40 µg/kg TNF-α caused a reduction of PVP after HAI by 32% (from 6.3 ± 0.9 to 4.0 ± 0.6 mm Hg), which recovered over the 120-min observation time. Comparable to control animals, resuscitation induced a striking increase of PVP in both TNF-α-treated groups (fig. 3).

Hepatic Microcirculation

In control animals, LDF revealed a constant blood flow in the hepatic microcirculation (zero time signal:

Fig. 4. Hepatic microcirculatory flux in percent of baseline (base) and during HAI as well as during the 120-min post HAI period and during the 30 min after HAES resuscitation (RES). HAI was performed either with NaCl (○) or 20 µg/kg TNF-α (●) and 40 µg/kg TNF-α (▲). Mean $\pm$ SEM; * $p < 0.05$ vs. NaCl controls.

200 ± 9.6 arbitrary units) during HAI as well as during the post-HAI observation period. Resuscitation showed no significant effect on microvascular blood flow as measured by LDF (fig. 4). In animals treated with 20 µg/kg TNF-α (zero time signal: 608 ± 79 arbitrary units) HAI caused a significant reduction of hepatic nutritive perfusion immediately after HAI. The microvascular flux remained decreased during the entire 120-min observation period, and could not be normalized by resuscitation with HAES ($p = 0.009$; fig. 4). In contrast, HAI with 40 mg/kg TNF-α (zero time signal: 464 ± 111 arbitrary units) showed no significant effect on the blood flow in the hepatic microcirculation as measured by LDF throughout the entire post-HAI observation period (fig. 4).

Discussion

In the present study, we investigated the effects of HAI with TNF-α on hepatic perfusion and microcirculation. The major finding of the present study is that HAI with TNF-α induces a slight and temporary decrease of PV flow which was associated with an overshooting hepatic arterial blood flow. As a consequence, HAI with TNF-α resulted in a 20–40% increase of total hepatic blood flow, which almost normalized during the 120-min observa-

tion period. Of interest, while high-dose (40 µg/kg) TNF-α application was not associated with significant blood flow disturbances of the hepatic microcirculation, the low-dose (20 µg/kg) TNF-α application resulted in a significant alteration of the nutritive liver perfusion.

As we have previously shown, HAI with TNF-α at a dose of 20 or 40 µg/kg body weight induces a slight depression of MAP, which is compensated by a reactive increase of HR rate [26]. This cardiovascular dysfunction after TNF-α administration is thought to be due to the induction of endothelium-derived vasoactive mediators such as nitric oxide [34]. Of interest, 20 µg/kg TNF-α has been shown to result in a hyperdynamic circulatory state, while a dose of 40 µg/kg did not affect cardiac index [26].

In the present study, we found that HAI with TNF-α reduces PV blood flow. This is most probably the consequence of the depression of the MAP. In general, reduced PV blood flow to the liver is compensated by increased arterial blood flow, a mechanism that is known as the ‘hepatic arterial buffer response’, thereby maintaining overall hepatic perfusion at a constant level [35]. Indeed, in our model, we observed a markedly increased hepatic arterial blood flow after HAI with TNF-α, resulting in hepatic hyperperfusion. As the hepatic arterial buffer response is adenosine-mediated [35], we could not prove that the observed increase of underlying hepatic arterial blood flow results from this mechanism. At least, it can be speculated that arterial hyperperfusion due to a TNF-α induced release of adenosine occurs (this release, however, does not seem to be dose-dependent), resulting in consecutive dysregulation of hepatic arterial blood flow. How far the underlying mechanism of hepatic hyperperfusion is adenosine-mediated, or may be due to other vasoactive mediators, remains to be determined in future studies.

Interestingly, the hepatic hyperperfusion was not dose-dependent because animals treated with 20 µg/kg TNF-α showed a more pronounced increase of hepatic arterial blood flow compared to animals treated with 40 µg/kg TNF-α. This may be due to the higher cardiac index observed after 20 µg/kg TNF-α compared to 40 µg/kg TNF-α application [26].

The hepatic arterial hyperperfusion did not substantially affect PVP. This is in line with findings by Lautt and Legare [36], demonstrating an autoregulation of PVP which keeps PVP stable independent of the PV blood flow.

One may assume that there was a low hematocrit throughout all experiments, influencing the measurements of PV and hepatic arterial blood flow. However, the

hematocrit values obtained in the present study were within the normal range known from the literature [37, 38]. A slight hemodilution may have occurred due to infusion of the animals by balanced anesthesia, but there was neither a difference in hematocrit between the experimental groups nor within one group throughout the duration of the experiment; therefore, the findings of the flow measurements may not be explained by the hematocrit values.

In the present study, the hepatic microcirculation was assessed by LDF, which is an established method for the estimation of the blood flow of the nutritive hepatic microcirculation [39, 40]. Interestingly, we found a reduced hepatic nutritive perfusion upon HAI only with low-dose TNF-α. Previous studies have shown that TNF-α induces endothelial injury with subsequent endothelial cell swelling, microvascular blood flow cessation, and thrombotic occlusion of sinusoids, reflecting well-known hallmarks of microvascular inflammatory injury [41–43]. However, this is probably not the cause for the reduced microcirculation observed after HAI with low-dose TNF-α because HAI with high-dose TNF-α did not affect the hepatic microcirculation and previous studies investigating HAI with 20 µg/kg TNF-α could not show thrombotic vascular occlusions, as already shown by previous research of our group [26]. Instead it may be speculated that the more pronounced hepatic hyperperfusion after low-dose compared to high-dose TNF-α application resulted in the deterioration of the microvascular perfusion. Although it has been demonstrated that the LDF signal from the liver reflects hepatic macrohemodynamics quite well [44], it is also known that a certain spatial and temporal heterogeneity of the LDF signal on the liver surface exists [40]. Especially in pathophysiological states such as low-flow states [27] or TNF-α application – which has never been examined by LDF – different perfusion patterns, intrahepatic shunts, or blood flow disturbances may occur [28]. This view is supported by other experiments, demonstrating in experimental small-for-size liver transplantation models that massive hepatic hyperperfusion is associated with a deterioration of the blood flow in the hepatic microcirculation [45, 46]. Of interest, after low-dose TNF-α application, the blood flow in the hepatic microcirculation did not recover during the later time course of the experiment, although the extent of hepatic hyperperfusion was found to be reduced. This observation parallels the observation of Li et al. [46], demonstrating sustained hepatic microcirculatory disturbances after portal hypertension lasting only 1 h in small-for-size liver transplantation.

Resuscitation with 250 ml HAES was effective to restore systemic hemodynamics, including normalization of both MAP and HR. This is in line with the established clinical practice using HAES to increase blood volume and to stabilize hemodynamic conditions [33]. Interestingly, resuscitation did not affect hepatic arterial perfusion, but substantially increased PV blood flow. This is most probably a result of the restored intestinal perfusion. The increased intestinal blood volume results in an increase of PVP, as observed in the present study in all groups.

TNF- α has a short half-life time of 14–18 min [47], and a high first-pass effect in the liver. Upon HAI, a large extent of the TNF- α is bound in the liver. As a consequence, systemic concentrations of TNF- α are low, avoiding major systemic side effects. In line with this view, our study demonstrates that the application of TNF- α to the HA is well tolerated without severe systemic side effects. Macro-circulatory parameters were only slightly influenced by the administration of TNF- α . This absence of systemic cardiovascular failure might be explained by a substantial TNF- α binding in the liver during the first pass of HAI.

In summary, we demonstrated herein that HAI of TNF- α induces a decrease of PV flow which is compen-

sated by an increase of hepatic arterial flow, which is not related to the dose of TNF- α applied. A lower dose of 20 $\mu\text{g/kg}$ body weight TNF- α leads to a higher hepatic arterial blood flow than the higher dose of 40 $\mu\text{g/kg}$ body weight, which may be explained by TNF- α related blood flow disturbances that are not dose-dependent. A slight overcompensation of the portal flow decrease by hepatic arterial hyperperfusion does not affect the blood flow in the hepatic microcirculation, while a more pronounced hepatic arterial hyperperfusion may impact hepatic microvasculature. In this study, we could demonstrate for the first time that an oncological effective dosage of TNF- α in nonisolated liver perfusion is feasible without severe systemic side effects. These findings could influence the therapy of patients with liver malignancies in order to perform hepatic perfusion without the need for vascular isolation of the liver.

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