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Pickering-emulsion for liver Trans-Arterial Chemo-Embolization with oxaliplatin

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Abstract

Purpose—Biodegradable polylactic-co-glycolic acid (PLGA) nanoparticles can adsorb at the water/oil interface to stabilize the emulsion (forming Pickering-emulsion). The purpose of this study was to compare the release profiles of oxaliplatin from Pickering-emulsion and Lipiodol-emulsion.

Materials/methods—Pickering-emulsions and Lipiodol-emulsions were both formulated with oxaliplatin (5mg/mL) and Lipiodol (Water/oil ratio: 1/3). For Pickering-emulsion only, PLGA-nanoparticles (15mg/mL) were dissolved into oxaliplatin before formulation. *In vitro* release of oxaliplatin from both emulsions was evaluated. Then, Oxaliplatin was selectively injected in left hepatic arteries of 18 rabbits bearing VX2 liver tumors using either 0.5 mL Pickering-emulsion (n=10) or 0.5 mL Lipiodol-emulsion (n=8). In each group, half of the rabbits were sacrificed at 1h and half at 24h. Mass-spectrometry was used to quantify drug pharmacokinetics in blood and resulting tissue (tumors, right- and left-livers) oxaliplatin concentrations.

Results—Pickering-emulsion demonstrated a slow oxaliplatin release compared to Lipiodol-emulsion ($1.5 \pm 0.2\%$ vs. $12.0 \pm 6\%$ at 1 h and $15.8 \pm 3.0\%$ vs. $85.3 \pm 3.3\%$ at 24h) during *in vitro* comparison studies. For animal model studies, the plasmatic-peak (Cmax) and the area under the curve (AUC) were significantly lower with Pickering-emulsion compared to Lipiodol-emulsion (Cmax= 0.49 ± 0.14 ng/mL vs. 1.08 ± 0.41 ng/mL, $p=0.01$ and AUC= 19.8 ± 5.9 vs. 31.8 ± 14.9 , $p=0.03$). This resulted in significantly lower oxaliplatin concentrations in tissues at 1h with Pickering-emulsion but higher ratio between tumor and left liver at 24h (43.4 vs. 14.5 , $p=0.04$).

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Conclusion—Slow release of oxaliplatin from Pickering-emulsion results in a significant decrease in systemic drug exposure and higher ratio between tumor and left liver oxaliplatin concentration at 24h.

Keywords

Emulsion; Lipiodol; Nanoparticles; Oxaliplatin; Liver; Hepatocarcinoma

Introduction

Drug delivery platforms are currently used during liver Trans Arterial Chemo-Embolization (TACE) procedures. These act as a transient reservoir, allowing slow release of the chemotherapy into the tumors' supply to achieve a prolonged exposure of the tumor cells while minimizing systemic exposures. In clinical practice, Lipiodol and drug eluting microspheres are the two most widely used drug delivery platforms for TACE. Both platforms have been used for the treatment of hepatocellular carcinoma¹ and liver metastases from neuroendocrine or colorectal cancers.

Lipiodol (Guerbet, Aulnay-sous-bois, France) is an oily based radio-opaque contrast agent that also serves as a drug delivery platform because of its radio-opacity and its preferential tumor uptake compared to surrounding liver tissue². Since most anticancer drugs are water-soluble, the vectorization mechanism consists in formulating a water-in-oil emulsion between the chemotherapy (aqueous-phase) and the Lipiodol (oily-phase)³. However, the release of chemotherapy occurs very rapidly because Conventional-emulsions have relatively poor stability. Within a few minutes, the two phases of the emulsion separate, with bulk of the water-soluble chemotherapy releasing rapidly from the Lipiodol resulting in high systemic exposures to the chemotherapy and diminished deposition of the chemotherapy into the targeted tumor tissues⁴.

Drug eluting microspheres are polyvinyl alcohol-based hydrogel microspheres that can be loaded with chemotherapy and used for TACE⁵. The presence of the anionic sulphonate group enables the sequestering of positively charged drugs only (Coulomb charge interactions). Thus, doxorubicin, epirubicin or irinotecan are the only chemotherapies that can be readily used in clinical practice. In addition, because of variable ionic interaction intensity between microspheres and the chemotherapy, the release could be either incomplete and very slow (doxorubicin) or complete and very fast (irinotecan)⁶. Finally, the microspheres are not only a drug delivery platform but also an embolic medical device (different sizes available, ranging from 40 up to 700 μm) that block the arterial flow. This property helps to increase the tumor drug exposure but can also induce hypoxia and the associated unwanted development of new tumor blood vessels (tumor neo-angiogenesis)⁷.

We have developed a new concept for a drug delivery platform that allows a slow and controlled release of various types of chemotherapies while maintaining the added value of Lipiodol in terms of a radio-opacity, preferential tumor uptake⁸ and transient vessels' occlusion⁹. The principle is based on Pickering technology^{10,11} that uses solid particles to stabilize emulsions. The use of solid particles (Pickering technology) in the formulation of an emulsion avoids the use of toxic synthetic surfactants and results in very stable interfaces

between the aqueous phase and the oily phase. The absence of synthetic surfactant makes Pickering-emulsion very attractive, especially for biomedical applications.

Oxaliplatin was the chosen chemotherapy in this study for two main reasons. First, several studies have already demonstrated that trans-arterial injection of platinum-based chemotherapy is effective in the treatment of unresectable liver tumors such as colorectal metastases^{12,13} and hepatocellular carcinoma^{14,15}. Second, few studies report the use of oxaliplatin-eluting microspheres in animal models¹⁶ and in a preliminary human study published in 2008¹⁷ but in clinical routine, no drug delivery platform for platinum-based chemotherapy can be used so far. Indeed, platinum-based chemotherapies cannot be loaded in drug-eluting microspheres since these chemotherapies are not positively charged. Few The use of Lipiodol is also problematic since the absence of powder form for platinum-based chemotherapies makes the reconstitution with contrast media impossible, resulting in very unstable emulsion. The purpose of this study was to compare the release profiles of oxaliplatin from Pickering-emulsion and Conventional-emulsion in both *in vitro* and *in vivo* settings.

Materials/methods

This study has two successive stages to compare Pickering-emulsion and Conventional-emulsion. The first stage was designed to compare the *in vitro* release profile of oxaliplatin from the two emulsions. The second stage used the VX2 rabbit liver tumor model to compare the plasmatic pharmacokinetics of oxaliplatin and tissue exposures upon release from the two emulsions.

Formulation of the Lipiodol-emulsions

Two different types of water-in-oil Lipiodol-emulsions were formulated: Conventional-emulsion and Pickering-emulsion.

For both emulsions, the oily-phase was composed of Lipiodol and the aqueous phase was composed of oxaliplatin (5 mg/mL). Polylactic-co-glycolic acid (PLGA) nanoparticles were added to the aqueous-phase during formulation of the Pickering-emulsion; latter component was the only difference between the 2 emulsions (15 mg of PLGA-nanoparticles per mL of oxaliplatin). These PLGA-nanoparticles are biodegradable¹⁸ and adsorb at the water/oil interface to stabilize the Lipiodol-emulsion (Figure-1). Water-in-oil Lipiodol-emulsions were formulated by continuous injection of 1 volume of aqueous phase in 3 volumes of oily phase with an electric syringe-pump (flow-rate of 1 mL per minute) during repetitive pumping of two 10-mL syringes through a three-way stopcock¹⁹.

In vitro stage

In vitro release of oxaliplatin from the two emulsions was evaluated. We performed the experiments twice for each Lipiodol-emulsion: an aliquot of 0.8 mL of emulsion was loaded into mini GeBAflex tubes (molecular weight cut-off: 12–14 kDa; Gene Bio-Application Ltd., Kfar Hanagide, Israel). These tubes were immersed in 40 ml of buffer solution (acetate buffer 25 mM, pH 6.8) and incubated in a shaking bath (37°C) rotated at a speed of 150 rpm

for the entire period. At predetermined times (1h, 4h, 24 h, 48h, 72h, 1 week, 2 weeks and 1 month), 0.5 ml of buffer solution were collected for oxaliplatin quantification.

Animal stage

This stage was performed in accordance with our institutional Animal Care and Use Committee guidelines. VX2 liver tumors were percutaneously implanted in New Zealand White rabbits under general anesthesia (Intramuscular injections of Ketamine 20–40mg/kg and xylazine 3–5mg/kg, Isoflurane 3–5% for induction and 1.5–3% for maintenance mixed with O₂ at 1L/min). For this purpose, 1–2 mm freshly harvested tumor fragments, coming from the hind limb tumors of donor rabbits were implanted under ultrasound guidance through an 18G needle positioned at 3 different locations of the left-liver. 1 week after the liver tumor implantations, MR checkup scans were performed to confirm tumor growth and locations within the liver.

Two weeks after liver tumor implantations, TACE procedures were performed by an experienced interventional radiologist (FD), under general anesthesia and fluoroscopic guidance (OEC 9800 Plus Mobile C-arm, Milwaukee, Wisconsin, USA). The femoral artery was surgically exposed and catheterized with a 4F vascular sheath using Seldinger approach. A micro-catheter (2.4F Merit Maestro Merit medical®, South Jordan, UT, USA) was used to selectively engage the left hepatic artery (Figure-2) and to inject 0.5 ml of emulsion corresponding to 0.625 mg of oxaliplatin. Attention was given to avoid any reflux into the right hepatic artery and additional embolization with gelatin sponge was considered only in cases of persistent flow in the left hepatic artery after completion of the emulsion injection.

Heparinized systemic blood samples (2 mL) were collected from the vascular sheath 5, 10, 20, 30 and 60 minutes after the injection of the emulsion to measure the plasmatic pharmacokinetics of oxaliplatin. The procedures were either non-recovery procedures (sacrifice at 1 hour, immediately after the last blood sample collection) or recovery procedures with sacrifice at 24 hours. Four different groups of rabbits were considered according to the type of emulsion each received and according to the delay before sacrifice:

- Group-1: rabbits received Conventional-emulsion and were sacrificed 1 hour after the injection.
- Group-2: rabbits received Pickering-emulsion and were sacrificed 1 hour after the injection.
- Group-3: rabbits received Conventional-emulsion and were sacrificed 24 hours after the injection.
- Group-4: rabbits received Pickering-emulsion and were sacrificed 24 hours after the injection.

Immediately after sacrifice, post-TACE MR scan (3T Magnetom Skyra; Siemens, Erlangen, Germany) using a 15 channels knee coil, was performed for each rabbit to measure and enumerate the liver tumors (Figure-2). Next, livers were resected. Tissues samples from the right- and left-livers, as well as all the tumors were dissected separately and homogenized with a blender for oxaliplatin quantification. Since the right-liver was not injected during

TACE, the quantification of oxaliplatin in this tissue was used to evaluate the systemic exposure of the chemotherapy after TACE.

Oxaliplatin quantification

Quantification of oxaliplatin was accomplished using inductively coupled plasma mass spectrometry (ICP-MS) of acid digested samples. Specifically, blood and tissue samples were digested in concentrated trace nitric acid (> 69%, Thermo Fisher Scientific, Waltham, MA, USA) and placed at 60 °C for 24 hours to allow for complete sample digestion. Ultra pure H₂O (18.2 MΩ·cm) was then added to produce a final solution of 3.0% nitric acid and 4.0% hydrochloric acid in a total sample volume of 5 mL. Platinum quantification served as our assay for oxaliplatin drug concentration (1 mg of oxaliplatin corresponds to 0.49 mg of platinum). Quantitative standards were made using individual 1000 µg/mL Platinum elemental standards (Inorganic Ventures, Christiansburg, VA, USA) which were combined to create a 100 ng/mL mixed element standard in 2.0% nitric acid and 4.0% hydrochloric acid (v/v) in a total sample volume of 50 mL. ICP-MS was performed on a computer-controlled (QTEGRA software) Thermo iCapQ ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) operating in STD mode and equipped with a ESI SC-2DX PrepFAST autosampler (Omaha, NE, USA). Internal standard was added inline using the prepFAST system. Online dilution was also carried out with the prepFAST system to generate calibration curves consisting of 100, 50, 20, 10, 5, 2, 1 ng/mL Platinum. Each sample was acquired using 1 survey run (10 sweeps) and 3 main (peak jumping) runs (40 sweeps).

Statistical Analysis

Data were presented as the mean value ± SD. Mann-Whitney test was used assess differences between means (t-test). P<0.05 was considered statistically significant.

Results

In vitro stage

The oxaliplatin release profiles of the two different emulsions are shown in Figure-3. Conventional-emulsion demonstrated a rapid drug release compared with Pickering-emulsion. The percentage of oxaliplatin released from Conventional-emulsions and Pickering-emulsions were respectively: 12.0 ± 6% vs. 1.5 ± 0.2% at 1 h, 50.0 ± 13.8% vs. 4.7 ± 0.9% at 4h, 85.3 ± 3.3% vs. 15.8 ± 3.0% at 24h. The release of oxaliplatin from the Conventional-emulsions was nearly complete within 24h whereas release was still on going from the Pickering-emulsions after 1 month (72.0 ± 2.1%).

Animal stage

A total of 18 rabbits (3.5 ± 0.3 kG) bearing a total of 33 VX2 liver tumors (1–3 tumor(s)/ rabbit) received selective left hepatic artery injection of 0.5 mL emulsion. No additional embolization with gelfoam was performed since forward flow was already completely stopped by the emulsion. Nine rabbits were sacrificed at 1 hour and 9 rabbits were sacrificed at 24 hours. Four rabbits bearing 6 tumors were included in Group-1, 5 rabbits bearing 9 tumors in Group-2, 4 rabbits bearing 9 tumors in Group-3 and 5 rabbits bearing 9 tumors in

Group-4. The maximal tumor diameter was 15.0 ± 6.4 mm without any statistically significant difference between the 4 groups.

Plasmatic pharmacokinetics of oxaliplatin—Plasmatic pharmacokinetics of oxaliplatin after injection of the two emulsions are shown in Table-1 and Figure-4. We observed a significantly lower peak oxaliplatin concentration in the blood (C_{max}) after injection of Pickering-emulsion (0.49 ± 0.14 ng/mL) compared to Conventional-emulsion (1.08 ± 0.41 ng/mL, $p < 0.01$). The AUC at 1 hour was significantly lower for the Pickering-emulsion (19.8 ± 5.9 vs. 31.8 ± 14.9 , $p = 0.03$).

Oxaliplatin concentration in liver and tumor tissues—Oxaliplatin concentrations in tissues (tumors, right- and left-livers) are respectively presented table-1 and in table-2. At 1 hour, we observed lower concentrations of oxaliplatin in liver and tumor tissues with Pickering-emulsion compared to Conventional-emulsion: right-liver (0.5 ± 0.2 vs. 1.3 ± 0.9 , $p < 0.01$), left-liver (0.6 ± 0.2 vs. 2.3 ± 1.4 ng/mg, $p < 0.01$) and tumor (17.6 ± 6.7 vs. 32.1 ± 21.2 ng/mg, $p = 0.02$). The concentration of oxaliplatin in tumors significantly decreased from 1 hour to 24 hours for both, Pickering-emulsion (17.6 ± 6.7 vs. 10.4 ± 9.8 ng/mg, $p = 0.01$) and Conventional-emulsion (32.1 ± 21.2 vs. 4.4 ± 4.8 ng/mg, $p = 0.001$). The concentration of oxaliplatin in left-livers significantly decreased from 1 hour to 24 hours for both, Pickering-emulsion (0.6 ± 0.2 vs. 0.3 ± 0.1 ng/mg, $p < 0.01$) and Conventional-emulsion (2.3 ± 1.4 vs. 0.4 ± 0.1 ng/mg, $p = 0.001$). The oxaliplatin concentrations in tumors at 24 hours were higher with Pickering-emulsion compared to Conventional-emulsions but difference was not significant (10.4 ± 9.8 vs. 4.4 ± 3.8 , $p = 0.08$).

At 24h, the oxaliplatin concentration ratio between tumor and the left-liver was significantly higher with Pickering-emulsion compared to Conventional-emulsion (43.4 ± 42.9 vs. 14.5 ± 6.6 , $p = 0.04$) and there was a trend of significance for higher ratio between the oxaliplatin concentrations in tumors and right-liver at 24 hours in favor of Pickering-emulsion compared to Conventional-emulsion (45.6 ± 38.5 vs. 18.6 ± 17.5 ng/mg, $p = 0.06$).

Discussion

The term “water-in-oil emulsion” refers to fine droplet dispersions of an aqueous-phase in a continuous oily-phase. The high interfacial energy of the dispersed droplets in the oily-phase contributes to thermodynamic instability and results in a coalescence process when droplets bump into each other and combine to form a larger droplet, so the average droplet size increases over time. However, a stable Lipiodol-emulsion is critical for TACE, allowing slow release of the chemotherapeutic agent into the liver tumor feeders for prolonged exposure, while minimizing systemic exposure^{20, 21, 22}. Stable emulsions can be obtained using substances known as emulsifiers that are able to decrease the interfacial tension between the two phases. Emulsifiers are commonly included in emulsion formulations. Synthetic surfactants have been used to stabilize emulsions made of Lipiodol and chemotherapy²⁰. However, these surfactants can raise toxicity issues, either directly or indirectly. Alternatively, the Pickering-emulsion uses solid particles as emulsifiers. The adsorption of the solid particles at the water/oil interface leads to the formation of a protective film that resists droplet coalescence and phase separation. The biodegradability of PLGA-

nanoparticles and the high stability of Pickering-emulsions lend unique properties when compared against classical emulsions stabilized by synthetic surfactants and make them very attractive for biomedical applications such as TACE.

We demonstrated that the use of PLGA-nanoparticles in the formulation of an emulsion of Lipiodol and oxaliplatin results in a slower release of oxaliplatin *in vitro*. Indeed, the release of oxaliplatin from a Conventional-emulsion was almost complete within 24 hours whereas only 15% of the oxaliplatin was released within 24 hours when nanoparticles were added into the aqueous-phase. Based on our *in vitro* study, we can also see that after 1 month, the total release of oxaliplatin from the Pickering-emulsion was roughly 72%. This slow release results in significant decrease of the plasmatic peak and of the area under the curve *in vivo*. The significantly higher concentrations of oxaliplatin in tumors and left-livers with Conventional-emulsion at 1 hour are consistent with the poor stability of the emulsion that results in a fast release of oxaliplatin from the Conventional-emulsion, as demonstrated *in vitro*. Conversely, the slow-release of oxaliplatin from the Pickering-emulsion resulted in significantly lower systemic exposure of “non-targeted” organs (right-livers) at 1 hour, with a trend for higher tumor exposure at 24 hours. In addition, Pickering-emulsion demonstrated a higher ratio between tumors and right-livers and between tumors and the left-livers at 24 hours, which is consistent with a stable water-in-oil emulsion. These pharmacological advantages provide interesting opportunities, either to decrease the systemic toxicities during TACE for similar amount of chemotherapy or to increase the amount of chemotherapy during TACE without additional systemic sides effects.

Oxaliplatin is a valid option for chemotherapy for patients suffering from HCC^{14,15} and colorectal cancer liver metastases^{12,13}. In colorectal cancer, oxaliplatin is a pillar of many systemic regimens in combination with 5-FU. Moreover the drug has been used through Hepatic Arterial Infusion (HAI) and its association with systemic chemotherapy has demonstrated very overall response rate in second or more advanced line of IV chemotherapy²³ and even in patients who have progressed on IV oxaliplatin²⁴. In rabbit VX2 liver tumor models²⁵, HAI of oxaliplatin has demonstrated a tumor versus healthy hepatic tissue ratio of 4.3 at 24h. Our reports a ratio at 24h of 44.1 for Pickering-emulsions and 11.8 for Conventional-emulsions, clearly suggesting the potential benefit of using a drug delivery platform for the arterial delivery of oxaliplatin in order to potentiate tumor response and reduce systemic toxicities.

We acknowledge some limitations to the current study. The first one was that we considered Conventional-emulsion as a gold standard for evaluating the release of oxaliplatin from Pickering-emulsions. However, there is no other drug delivery system that we could have used for comparison since oxaliplatin cannot be loaded onto clinically approved drug eluting bead platforms. A high ratio of Lipiodol/oxaliplatin (3/1) and a continuous and slow incorporation of oxaliplatin in the Lipiodol were considered in order to obtain the best stability of the Lipiodol-emulsion^{3, 26, 19}. Another limitation was that we decided to early sacrifice the rabbits in order to demonstrate a pharmacokinetic advantage within the first 24 hours. Since, oxaliplatin slowly released from Pickering emulsion, additional animals must be done to evaluate histological findings (necrosis) and tumor response. Another limitation was that no embolic agent was injected during the TACE procedures (as would commonly

be performed for clinical TACE studies). However, hepatic artery antegrade flow was completely stopped at the conclusion of each emulsion injection thus additional embolization would have sub-optimally resulted in retrograde-flow within extra-hepatic arteries in our rabbit model. Additional embolizations would also introduce biases during the evaluation of oxaliplatin release from the emulsions. Finally, additional studies must be done to further evaluate the biocompatibility of the PLGA-nanoparticles that we used in this study.

We believe that this experimental study may offer potential implication for patient care. First, new chemotherapies, such as oxaliplatin, can be stabilized in Lipiodol-emulsion thanks to the use of nanoparticles, offering new perspectives for liver TACE. The second point of interest is that the use of nanoparticles significantly decreases systemic oxaliplatin exposure and increases the tumor oxaliplatin concentration selectivity at 24h. This could lower systemic toxicities after Lipiodol-TACE and potentially increase tumor response in liver cancer patients.

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Figure-1. Confocal scanning laser microscopy of Pickering-emulsion. Nanoparticles appears in blue (rhodamine) and surrounded the dispersed aqueous droplets in the continuous oily-phase (Lipiodol), confirming that nanoparticles adsorbed at the interface to stabilize the emulsion.

Figure-2.

Trans-Arterial Chemo-Embolization (TACE) with oxaliplatin in a New Zealand White rabbit: angiography in the celiac trunk (A) and in the left-hepatic artery (B), post-TACE MR scan (C) and resected liver (D) showing a 15 mm large VX2 liver tumor (white arrow).

Figure-3.
In-vitro release of oxaliplatin from the Lipiodol-emulsion and from Pickering emulsion: percentage released (A) and masse released (B).

Figure-4. Oxaliplatin plasmatic pharmacokinetics after injection of the emulsions in the left hepatic arteries.

Table 1

Mean oxaliplatin concentration (ng/mg) in liver tissues according to the type of emulsion injected and according to the delay before sacrifice.

	Emulsions:		
	<u>Conventional</u>	<u>Pickering</u>	
• Pharmacokinetic	<i>n= 8 rabbits</i>	<i>n= 10 rabbits</i>	
C max (ng/mL)	1.08 ± 0.41	0.49 ± 0.14	p< 0.01 *
AUC	31.8 ± 14.9	19.8 ± 5.9	p= 0.03 *
• Sacrifice at 1 hour	<i>n= 4 rabbits (group-1)</i>	<i>n= 5 rabbits (group-2)</i>	
Oxaliplatin	2.3 ± 1.4	0.6 ± 0.2	p< 0.01 *
- Left-liver			
- Right-liver	1.3 ± 0.9	0.5 ± 0.2	p< 0.01 *
- ratio left/right-liver	1.8 ± 0.7	1.1 ± 0.2	p= 0.07
• Sacrifice at 24 hours	<i>n= 4 rabbits (group-3)</i>	<i>n= 5 rabbits (group-5)</i>	
Oxaliplatin	0.4 ± 0.1	0.3 ± 0.1	p= 0.04 *
- Left-liver			
- Right-liver	0.2 ± 0.4	0.2 ± 0.03	p= 0.5
- ratio left/right-liver	1.7 ± 0.4	1.4 ± 1.0	p= 0.2

* significant difference.

Table 2

Mean oxaliplatin concentration (ng/mg) in liver tumors according to the type of emulsion injected and according to the delay before sacrifice.

	Emulsions:		
	<i>Conventional</i>	<i>Pickering</i>	
• Sacrifice at 1 hour	<i>n= 6 tumors (group-1)</i>	<i>n= 9 tumors (group-2)</i>	
Maximal tumor diameter (mm)	14.1 ± 11.1	16.0 ± 5.9	p= 0.6
Oxaliplatin:	32.1 ± 21.2	17.6 ± 6.7	p= 0.02 *
- Tumors			
- ratio tumor/left-liver	18.0 ± 10.8	33.2 ± 14.9	p= 0.07
- ratio tumor/right-liver	29.1 ± 11.4	40.3 ± 19.3	p= 0.3
• Sacrifice at 24 hours	<i>n= 9 tumors (group-3)</i>	<i>n= 9 tumors (group-4)</i>	
Maximal tumor diameter (mm)	15.0 ± 2.9	14.5 ± 5.0	p= 0.4
Oxaliplatin:	4.4 ± 3.8	10.4 ± 9.8	p= 0.08
- Tumors			
- ratio tumor/left-liver	11.8 ± 8.5	44.1 ± 43.3	p= 0.02 *
- ratio tumor/right-liver	18.6 ± 17.5	45.6 ± 38.5	p= 0.06

* significant difference.