Experiments with zenCELL owl® Live Cell imaging system

All experiments were done in the Department of General, Visceral and Transplant Surgery, Labs of Experimental Oncology, University of Tübingen, Tübingen, Germany, by Ms. Lisa Brauer, MD and Mr. Jürgen Weinreich, PhD.

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Introduction:

Peritoneal metastasis (PM) is a major reason for poor prognosis and mortality in gastric cancer (GC) and colorectal cancer (CRC). In fact, the peritoneum is the second most affected site of metastasis in CRC after the liver and malignant cells in the peritoneal cavity have been reported for 22% of CRC and 6% of GC patients. Different to primary tumors, which can be easily targeted by cytostatic drugs via the blood vessels, PM have been shown to be highly resistant to systemic chemotherapy which is partly due to the peritoneal blood barrier. This is why cytoreductive surgery or intraperitoneal drug administration are some of the few therapeutic options so far, that were shown to have a measurable curative effect. A detailed and holistic look at the peritoneal environment in GC and CRC would help to understand why current treatment approaches are failing and it is important to develop new therapies. Therefor the aim of our experiments is to investigate a new combination of two cytostatic agents for the therapy of peritoneal metastasis from primary gastric intraperitoneal tumors and to test this combination in a 2D cell culture model with MKN-45 gastric cancer cells (representing PM from gastric cancer) and MeT-5A normal mesothelial cells (representing the effect to healthy peritoneum tissue). For the experiments we used the microscope zenCell owl® scratch assay technology from OLS company.

Cell treatment:

For all experiments a standard 24-well microtiterplate (greiner bio one, CELLSTAR® , Cat.No. 662 160) was used. MKN-45 cells were seeded at a concentration of 2 x 10^6 / well whereas MeT-5A were seeded at 1,6 x 10^6 / well and let growth to 100% confluence. Standardized scratches were done with a sterile 10 µl pipette tip in a middle vertical position. The old medium was aspirated and new medium complemented with the appropriate drugs were added. The cells were than incubated for 30 min in an incubator at 37° C and 5% CO_2. After drug treatment the liquid supernatant was aspirated and a fresh growing medium without drugs was added. The cells were incubated again and cell migration activity in the scratch region was monitored over 72 hours using the incubator microscope zenCell owl®. With an image capturing interval of 10 min a short video of each well could be recorded over three days.

Drug combination:

Until now, chemotherapeutic drugs like the cytostatic agent Paclitaxel (PTX) have been used for preventing peritoneal metastasis after surgery for gastric cancer. But these chemotherapeutic drugs impair wound healing. Due to this fact, we investigated valproic acid (VPA) as a possible agent with synergistic effects to cancer cells when used with PTX. VPA is known as a well tolerated antiepileptic drug and is also an inhibitor of the histone deacetylase enzymes (HDAC). HDACs play an essential role in regulating gene expression and therefor probably in carcinogenesis. So VPA has multidimensional effects on several cancer hallmarks like tumor growth, metabolism, adhesion, and migration and additional VPA does not seem to impair wound healing. Because studies have demonstrated a chemosensitizing effect of VPA in combination with chemotherapeutic agents we want to investigate a possible synergistic antitumoral effect for a combined drug regimen of VPA and PTX . Prior to the combination we investigated the effect of following solutions to the mentioned two cell lines in touch for tumor inhibition and wound healing inhibition effects:

Group 1: 0,9% NaCl solution (control)

Group 2: PTX; 3 µg/ml

Group 3: VPA; 252 µg/ml

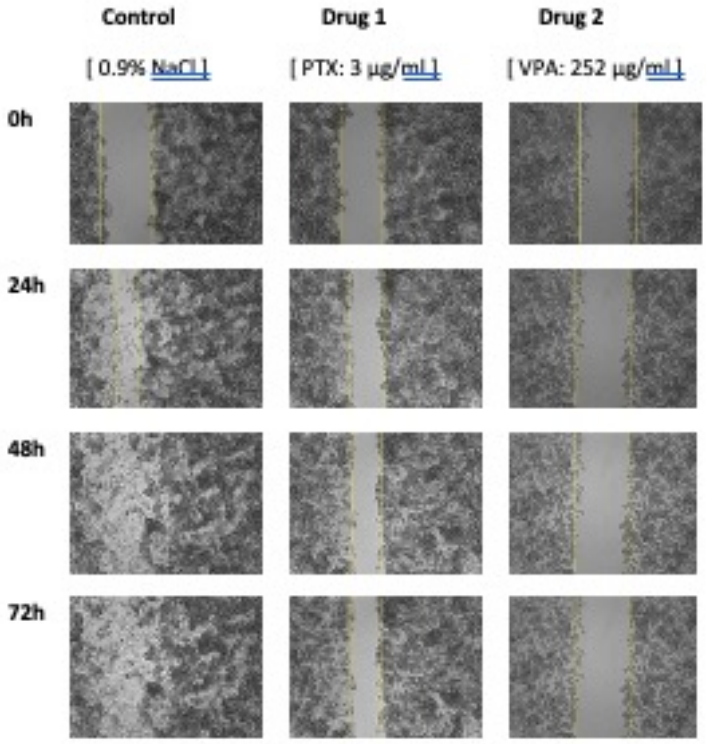
Calculations of scratch area:

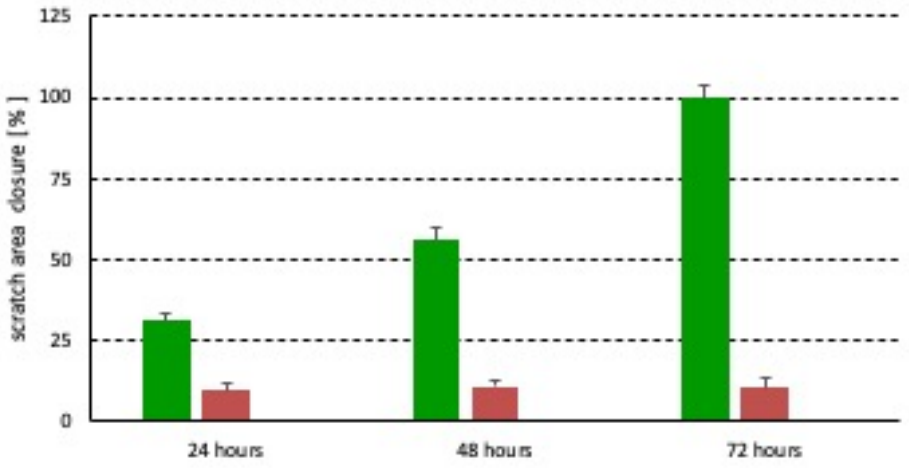
Scratch area closure in percent were calculated with image processing software ***imageJ*** (Wayne Rasband, 1997). Scratch cell free area at 0h were set to 0 %, whereas cell covered area at 24h, 48h and 72h were set to the appropriate values. Statistical analysis were calculated with office 10 excel software.

Experiment 1:

Scratch assay with MKN-45 [ATCC®] human gastric cancer cells under influence of different drugs over 72 h

view of the well history window



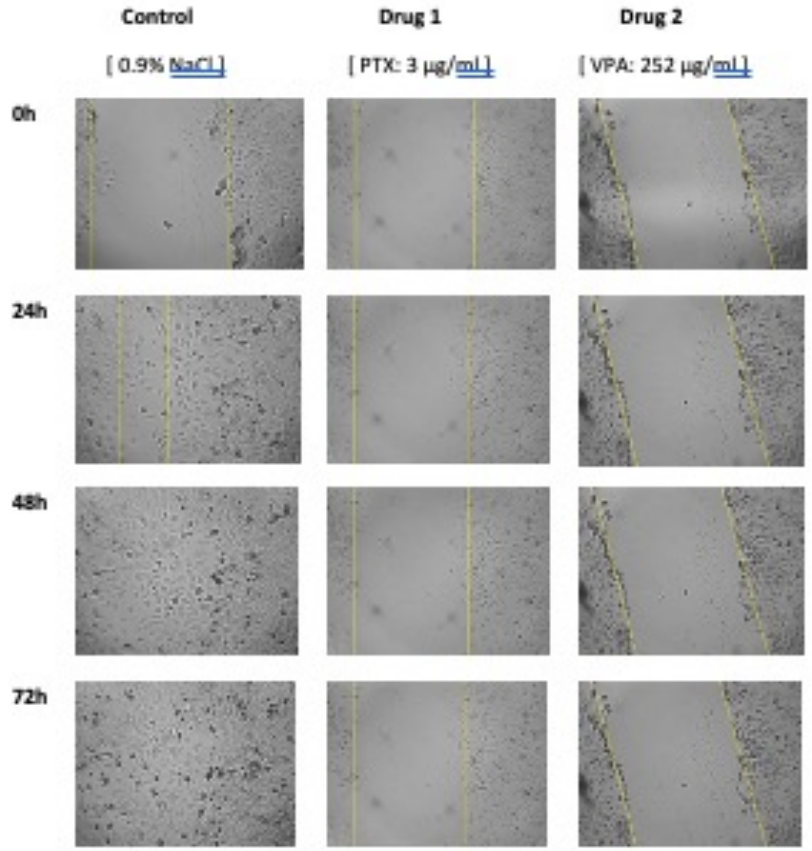


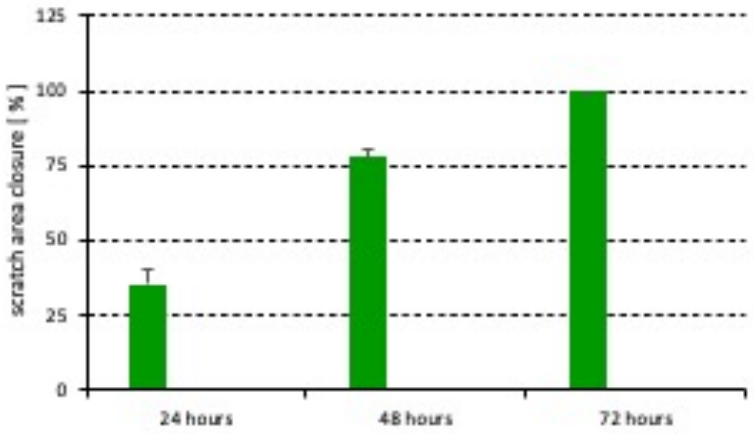
control [0.9% NaCl] ; Drug 1 [PTX: 3 µg/ml] ; Drug 2 [VPA: 252 µg/ml]

Experiment 2:

Scratch assay with MeT-5A [ATCC®;CRL-9444™] normal human mesothelial cells under influence of different drugs over 72 h

view of the well history window





control [0.9% NaCl] ; Drug 1 [PTX: 3 µg/ml] ; Drug 2 [VPA: 252 µg/ml]

Cell coverage example

Cell coverage graph of control group Met-5A cells treated with 0,9% NaCl solution over 72 h

Results:

The result analysis of the experiments showed a significant inhibition of the scratch closure for MKN-45 gastric cancer cells as well for MeT-5A mesothelial cells when treated with either 3 µg/ml PTX or 252 µg/ml VPA for 30 min versus the 0.9 % NaCl control over a time period of 72 h. Unfortunatly we have a negative effect on mesothelial cells which represent the normal peritoneal tissue. In fact of this result possible wound healing inhibition effects can not be excluded. Further experiments especially with MeT-5A cells and a treatment concentration of VPA which is lower as 252 µg/ml as well as 3 µg/ml PTX have to be done to eliminate the unwanted wound healing inhibition effects.

Conclusion:

The zenCELL owl® Live Cell imaging system from OLS is a proper tool for the analysis of different new drug regimen used in gastrointestinal malignant tumor therapies.

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