Effect of pantethine on ovarian tumor progression and choline metabolism

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Introduction
Epithelial ovarian cancer remains the leading cause of death from gynecologic malignancy among women in developed countries with an estimated incidence of 205,000 cases worldwide per year, resulting in 125,000 deaths. Although the prognosis in cases detected at an early stage is quite favorable, the vast majority of cases are diagnosed at an advanced stage when five-year survival rates are only 30-40%. The poor prognosis of ovarian cancer is due to a combination of the aggressive characteristics of the disease and a lack of effective therapy, further compounded by late detection and resistance of most relapsed tumors to current treatments. Metastases and malignant ascites are complications frequently observed in ovarian cancer at the time of diagnosis. New therapeutic strategies are urgently needed to minimize morbidity and improve survival rates for ovarian cancer patients. To evaluate treatment strategies it is important to use models that closely mimic tumor growth in humans. We therefore used an orthotopic model of ovarian cancer where a piece of tumor tissue, derived from an ovarian tumor xenograft, is engrafted directly onto the ovary of female mice, to maintain the tumor physiological environment. The model frequently results in metastases and malignant ascites. The orthotopic tumor growth can be followed non-invasively with MRI. In the present study, we investigated the effect of pantethine as a therapeutic agent in an orthotopic model of ovarian cancer. Pantethine, the precursor of coenzyme A, has multifaceted activities. In a mouse model, pantethine has been shown to prevent the perivascular inflammation associated with cerebral malaria (1). Since pantethine is known to inhibit fatty acid synthase (2), and phosphatidylcholine (PtdCho) synthesis (3), we also assessed the effect of the treatment on tumor metabolism, by performing high-resolution 1H magnetic resonance spectroscopy (MRS) on tumor extracts.

Methods
Surgical orthotopic implantation of OVCAR3 tumor was performed by engrafting a piece of tumor tissue onto the ovary of severe combined immunodeficient (SCID) female mice. Tumor tissue pieces were obtained from subcutaneous tumors after inoculation of 2 x 10^6 cells in the flank of female SCID mice. Treatment with pantethine started when tumors were approximately 100 mm^3, and consisted of a daily i.p. injection at a dose of 750 mg/kg. Under these conditions, no side effects were observed. A control group was injected daily with saline. Tumor growth was followed non-invasively by MRI on a 4.7T Bruker Avance spectrometer using a home-built volume coil. Mice were anesthetized with an i.p. injection of ketamine and acepromazine. T1-weighted images and diffusion-weighted images were acquired to localize the tumors and measure their volume. High-resolution 1H MRS was performed on tumor extracts. A dual-phase extraction method based on methanol/chloroform/water was used to obtain lipid and water-soluble fractions from the tumors (4). Fully relaxed 1H MR spectra of tumor extracts were acquired on an 11.7T spectrometer using a 5-mm HX inverse probe and the following acquisition parameters: 30° flip angle, 6000 Hz sweep width, 12.7 s repetition time, time-domain data points of 32K, and 128 transients. Spectra were analyzed using Bruker XWIN-NMR 3.5 software. Integrals of the metabolites of interest were determined and normalized to tumor weight. Metabolite concentrations were obtained from 1H spectra using an internal standard.

Results and Discussion
Here we used an orthotopic model of ovarian cancer that mimics human disease; we observed peritoneal invasion by ovarian cancer cells, malignant ascites formation, metastases in the liver and on the diaphragm, and seeding in the abdominal cavity. We followed tumor progression non-invasively with MRI during the treatment period. We observed significantly reduced tumor growth in the pantethine treated group compared to the control group (Figure 1). Interestingly, we found liver metastases in 86% of the control mice, but only in 43% of the treated mice. Lung metastases occurred in 29% of the control mice, but not in treated mice, and malignant ascites was present in 86% of control mice, but only 29% of the treated mice (n = 7) (Figure 2). To assess the effect of the treatment on tumor metabolism, we performed dual phase extraction of treated and control tumors 4 weeks after treatment. The lipid phase analysis revealed a 38% reduction of PtdCho in the treated tumors compared to control tumors (n = 5, p < 0.05) (Figure 3). We also observed a 35% reduction of phosphocholine (PC) in the treated tumors (Figure 4). Our study identifies pantethine as a promising new drug against ovarian cancer, that impacts tumor progression, choline metabolism, metastases occurrence, and ascites formation. PC has been shown to be increased in ovarian cancer cells compared with normal and nontumoral immortalized cells (5), and a drop in PC was observed following treatment with a fatty acid synthase inhibitor (6), consistent with a decrease of PC observed following treatment with pantethine. Mechanistic studies on the effects of pantethine on choline metabolism, on tumor progression, and on metastases formation are ongoing.

References