Carbogen breathing differentially enhances blood plasma volume and 5-fluorouracil uptake in two murine colon tumor models with distinct vascular structures

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Introduction: For the systemic treatment of colorectal cancer 5-fluorouracil (FU)-based chemotherapy is standard. However, only a subset of patients respond to chemotherapy. Breathing of carbogen (95% O_2; 5% CO_2) may increase uptake of FU by changes in tumor physiology. The aim of this study was to monitor *in vivo* in animal models the effect of carbogen breathing on tumor blood plasma volume, pH and energy status, as well as the effect on FU uptake and metabolism in C38 and C26a colon tumors that show profound differences in vasculature structure.

Materials and Methods: C38 and C26a murine colon tumor tissue fragments were implanted subcutaneously in female C57BL/6 and BalbC mice, respectively. Experiments started when tumors reached a maximum diameter of approximately 8 mm ^{31}P magnetic resonance spectroscopy (MRS) was used to assess tumor pH and energy status and ^{19}F MRS to follow FU uptake and metabolism. ^{31}P and ^{19}F MRS measurements were interleaved, using a home-made double tuned coil. Experiments were performed on a S.M.I.S. 7T horizontal bore animal system. An unlocalized ^{19}F/^{31}P interleaved pulse-acquire sequence (^{31}P:^{19}F = 1:6) with a 90° rectangular RF pulse of 20μs was used in all experiments. TR for ^{31}P MR aquisition was 3 sec with a total acquisition time of 4 min. In ten mice with a C38 tumor and 12 mice with a C26a tumor the breathing gas was switched to carbogen 5 min before start of the ^{31}P/^{19}F MRS measurements and continued with carbogen during 28 min, i.e. during the first seven measurements. Then FU was administered (150 mg/kg) i.p. within ten seconds before start of the first ^{31}P/^{19}F MRS measurement. Twelve mice with a C38 tumor and ten mice with a C26a tumor did not undergo carbogen breathing and served as a control. As a measure of relative blood plasma volume, inversion recovery snapshot fast low-angle shot (IR-FLASH) was performed to measure the water T_1 relaxation relaxation rate of water proton spins, after intravenous administration of a contrast agent which consisted of ultra small superparamagnetic particles of iron oxide (USPIO). T_1 relaxation times were measured during 1 h after administration of USPIO at five time points (cf. fig. 2). In six mice with a C38 tumor and six mice with a C26a tumor carbogen breathing was started 13 min after injection of sinerem, during 30 min. Three mice with a C38 tumor and four mice with a C26a tumor did not undergo carbogen breathing and served as a control.

Results and Discussion: Carbogen breathing significantly decreased pHe (fig. 1), increased tumor blood plasma volume (fig. 2), and increased FU uptake in both tumorlines. These effects were most significant in the C38 tumor, which may be due to a difference in responsive tumor vasculature in C38 and C26a tumors.[1] It has been hypothesized that an increase in tumor blood flow may in itself be insufficient for an increased uptake of FU in solid tumors, since not only drug delivery but also drug clearance will be increased.[2] Therefore, for carbogen to increase FU uptake a secondary mechanism may be necessary, apart from an increase in tumor blood flow. For example a decrease in pHe, as we observed, which is associated with an increase FU uptake in tumors. In fact, we observed an increased FU uptake in both tumorlines. However, carbogen breathing also enhanced systemic toxicity by FU and increased mortality in the carbogen breathing group. In previous studies no toxicity has been described after the combination of FU treatment with carbogen breathing. Not only the administered dose of FU, but also the duration of carbogen breathing may be crucial for the occurrence of systemic toxicity. Increased clearance of FU from the plasma was observed after 30 min of carbogen breathing compared to 20 min of carbogen breathing.[2] In conclusion, even within one tumor type, namely colon carcinoma, the effect of carbogen breathing on tumor physiology and FU uptake and metabolism differs, which may be caused by differences in tumor vasculature. An increase in FU uptake in the tumors was observed after carbogen breathing, but systemic toxicity was also enhanced. Thus, a clinical study evaluating the effect of carbogen breathing on FU efficacy should be preceded by a phase I trial monitoring the effect of carbogen breathing on FU toxicity.

[1] Van Laarhoven et al., *IJROBP* 2004 (60) 310-321.
[2] Mc Sheehy et al., *Cancer Chemother Pharmacol* 2005 (55) 117-128.

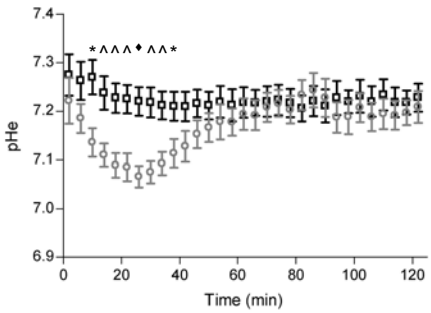

Figure 1 Extracellular pH (pHe), for C38 tumors of the control ■ and carbogen breathing ○ groups. The error bar indicates the standard error of the mean. A significant difference between control and carbogen breathing group is indicated by * p < .05; ^ p < .01; ♦ p < .001. Carbogen breathing is started at t = -5 min and terminated at t = 28 min; FU is injected at t = 0.

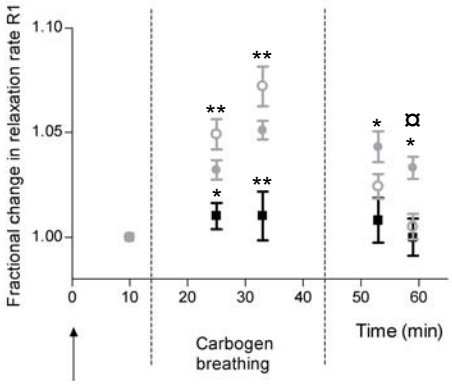

Figure 2 Fractional change in the longitudinal relaxation rate R1, which is proportional to plasma blood volume, for C38 and C26a tumors 3 min before start carbogen breathing, during carbogen breathing and after terminating carbogen breathing. The error bar indicates the standard error of the mean.
* p < 0.05 (control and carbogen breathing)
** p < 0.01 (control and carbogen breathing)
¤ p < 0.01 (C38 and C26a tumors)

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