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Effect of anesthesia on the signal intensity in tumors using BOLD-MRI: Comparison with flow measurements by laser Doppler flowmetry and oxygen measurements by luminescence-based probes

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Abstract

BOLD-contrast functional magnetic resonance imaging (MRI) was used to assess the evolution of tumor oxygenation and blood flow after administration of four different anesthetics: pentobarbital (60 mg/kg), ketamine/xylazine (80/8 mg/kg), fentanyl/droperidol (0.078/3.9 mg/kg), and isoflurane (1.5%). Gradient echo sequences were carried out at 4.7 Tesla in a TLT tumor model implanted in the muscle of NMRI mice. In parallel experiments, tumor blood flow and tumor pO_2 were measured using the OxyLite/OxyFlo probe system. A comparison was made with the changes occurring in the skeletal muscle (host tissue). The signal intensity was dramatically decreased in tumors after administration of anesthetics, except isoflurane. These results correlated well with measurements of oxygenation and blood perfusion. Isoflurane produced constant muscle pO_2 and blood perfusion although large transient fluctuations in pO_2 and blood flow were reported in some tumors. Our results emphasize the need for careful monitoring of the effects of anesthesia when trying to identify new therapeutic approaches that are aimed at modulating tumor hemodynamics.

Keywords: BOLD MRI; Anesthesia; Tumor; Blood flow; Oxygen

1. Introduction

Oxygen is a key environmental factor in the development and growth of tumors, and in their response to treatments. Various methods have been developed to monitor changes in tumor perfusion and/or oxygenation after treatments. Among these, functional magnetic resonance imaging (fMRI), based on the blood oxygen level dependent (BOLD) effect offers a way to localize functional changes after a specific treatment. Several groups have used gradient-echo (GRE) MRI to monitor the tumor response to high-oxygen-content gases [1,2] or to vasoactive agents [3,4]. In tumors, the BOLD effects observed in two-dimensional (2D) GRE images have been attributed to changes in both blood flow and blood oxygenation [5]. Although not quantitative, the BOLD signal response is temporally correlated with changes in pO_2 [6].

Research for new types of interventions to modulate the tumor oxygenation is generally conducted in rodents. Anesthetics are usually required in these studies. Ideally, anesthesia itself should exert little or no effect on normal tissue or tumor homeostasis. However, many of the commonly used anesthetics can markedly influence tumor blood flow [7,8], radiosentivity [9–13], or the uptake and distribution of anticancer drugs [14]. Previous investigations assessed either the perfusion or the oxygenation status of the tumor tissue, mostly with poor temporal resolution. The effect of anesthetics on blood perfusion in rodent tumors has been investigated by a variety of techniques such as radioisotope clearance techniques [7,8], thermodynamic methods [15], or deuterium (2H) nuclear magnetic resonance [16]. The effect of anesthetics at the level of tumor oxygenation, which is a more relevant parameter in terms of radiosentivity, has also been characterized by near infrared spectroscopy [13] or using polarographic methods [17]. Continuous measurements of tumor pO_2 after administration of anesthetics has been performed recently using the time-resolved luminescence-based optical OxyLite probe [18].

Because anesthesia can interfere with the basal flow and

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pO_2 in tumors, and because of the increased application of BOLD-MRI to characterize changes in tumor hemodynamics, it is important to assess the influence of anesthesia on tumors using this imaging technique. In the present study, the effects of four different anesthetics, ketamine/xylazine, pentobarbital, fentanyl/droperidol, and isoflurane, at recommended doses were monitored in murine tumors using 2D GRE MRI. In parallel experiments, the OxyLite/OxyFlo system (Oxford Optronix, Oxford, UK) was used to evaluate tumor pO_2 and microvascular red cell blood flow simultaneously [19,20]. Time-dependent changes in pO_2 and blood flow inside the tumor tissue were dynamically tracked and compared with those occurring concomitantly inside its host tissue (skeletal muscle).

2. Materials and Methods

2.1. Tumor model

Male NMRI mice (Animalerie Facultaire, Faculty of Medicine, Catholic University of Louvain, Brussels) were used. The experimental tumors were grown intramuscularly after injection of ascites cells of a transplantable mouse liver tumor model (TLT) into the right gastrocnemius muscle [21]. Tumors were used when they reached 8 mm in diameter (7–8 days after inoculation).

2.2. Drugs

Ketamine/xylazine (80 mg/kg ketamine [Imalgène, Merial, Lyon, France], 8 mg/kg xylazine [Rompun, Bayer, Brussels, Belgium]) was administered (n = 9) intraperitoneally (ip). Pentobarbital (Nembutal, Sanofi, Brussels, Belgium) was administered (n = 6) ip at a dose of 60 mg/kg. Fentanyl/droperidol (Thalamonal, Janssen-Cilag, Beerse, Belgium) was administered (n = 9) ip (78 μg/kg fentanyl, 3.9 mg/kg droperidol). Isoflurane (Forène, Abbott, Louvain-La-Neuve, Belgium) was delivered using a calibrated vaporizer at 1.5% in air and administered via a facemask (n = 6).

In order to maintain anesthesia for at least 1 h, additional drug applications were given as follows: ketamine (25 mg/kg) every 15–20 min, pentobarbital (60 mg/kg) every 40 min, fentanyl (78 μg/kg)-droperidol (3.9 mg/kg) every 25–30 min.

2.3. OxyLite-OxyFlo

Probe implantation and placement of the catheter for ip drug administration were conducted under isoflurane anesthesia. First, anesthesia was induced using 3% isoflurane. After 5 min induction, isoflurane was decreased to 2% for insertion of fiber-optic sensors and catheter placement. A combined fiber-optic probe (~500 μm outer diameter, OxyLite/OxyFlo instruments, Oxford Optronix Ltd., Oxford, UK) was introduced into the tumor and into the contralateral muscle, providing real-time and simultaneous measurement of tissue pO_2 (by luminescence quenching), microvascular erythrocyte flow (by laser-Doppler flowmetry) and temperature. The probe was moved into the tumor until a clearly nonzero pO_2 was obtained, so that the measurement was carried out in a non-necrotic area of the tumor and the pO_2 had the opportunity to either increase or decrease.

This step lasted 15–20 min, then isoflurane was decreased to 1.5%. After waiting ~10 min for sensor equilibrium and stabilization, a 10-min baseline measurement was recorded. Thereafter, the anesthetic agent was administered (~200 μL) while isoflurane was disconnected from inspired air. To prevent hypothermia, body temperature was regulated using a thermostatically controlled heated pad (Homeothermic blanket control unit, Harvard Apparatus Ltd, Kent, UK).

Data were collected continuously at a sampling frequency of 20 Hz Laser-Doppler signals were recorded in blood perfusion units, which is a relative units scale, and tissue pO_2 in mm Hg.

Data were saved and 3-min averages were computed. Microvascular red cell flow data were further transformed to obtain relative changes, whereas the temporal evolution of the pO_2 was reported in mm Hg. Relative change in blood flow at time i was calculated as follows:

$$rBF = \frac{S_i}{S_0} \quad (1)$$

with S_0, being the first 3-min average calculated under isoflurane 1.5%, and S_i the 3-min average signal at time i.

For each anesthetic regimen, an average time series was calculated. One-way analysis of variance and Dunnett posttest were performed to compare pO_2 values under different anesthetics (pooled pO_2 values from 10 min post-injection to the end of the experiment, i.e., 40–60 min averaging) vs. those under isoflurane; 95% Confidence intervals for the average percent change of blood flow (pooled values from 10 min post-drug administration to the end of the experiment) were calculated for the four anesthetic regimens.

2.4. T_2*-weighted gradient echo MRI

MRI was performed with a 4.7 Tesla (200 MHz, 1H) 40-cm inner diameter bore system (Bruker Biospec, Ettlingen, Germany). A surface coil 2 cm in diameter was used for radiofrequency transmission and reception. Isoflurane (1.5%)-anesthetized mice were secured using adhesive tape to prevent movement artifacts. Warm air was flushed into the magnet to maintain body temperature around 37°C. Anatomical images were acquired using a fast spin echo sequence (repetition time (TR) = 3 s, effective echo time (TE) = 63 ms, 3 cm field of view [FOV]). A 1-mm slice passing through the tumor center was selected. The T_2*-

weighted gradient echo (T_2*w GRE) sequence used the following parameters: TR = 200 ms, TE = 25 ms, flip angle = 45°, 12.5 kHz receiver bandwidth, 64 phase and frequency encode steps, 2 averages, 3 cm FOV, resulting in an acquisition time of 25.6 s. Images were acquired continuously during ~51 min (120 scans). After a 10-min baseline acquisition, the anesthetic agent was administered (~200 μl), while isoflurane was disconnected from inspired air. Viewing the series of images in cine mode ensured absence of leg motion. "Whole-tumor" (single slice) average relative changes in signal intensity (rSI) were reported.

3. Results

3.1. T_2*-weighted MRI

Evolution of the T_2*w GRE MRI signal for the tumor tissue is shown in Fig. 1. Ketamine/xylazine caused a rapid and long-lasting decrease in SI (n = 3). A fall in SI (but less steep) was also observed following fentanyl/droperidol (n = 3) and pentobarbital administration (n = 2). SI remained fairly constant under isoflurane anesthesia, but some transient signal fluctuations were also observed (n = 2). Unlike the whole tumor average analysis, a voxel-by-voxel analysis revealed some local heterogeneity in the tumor response. Indeed, some tumor regions displayed opposite patterns of response. For example, in ketamine/xylazine mice, for some voxels inside the tumor, an increase in SI was observed following drug injection instead of a decrease.

3.2. OxyLite/OxyFlo

3.2.1. Tissue temperature

The muscle and tumor temperature was effectively maintained between 31° and 33°C throughout the experiment.

3.2.2. Oxygen partial pressure

In a preliminary study, skeletal muscle pO_2 was measured under ketamine/xylazine anesthesia with or without isoflurane preanesthesia. No difference in muscle pO_2 values was observed between the two experimental conditions (with isoflurane: 15.2 ± 7.3 mm Hg (n = 9); without isoflurane: 15.5 ± 6.0 mm Hg (n = 9), mean ± SD, $p > 0.9$, *t*-test). This indicated that the anesthesia induction with isoflurane (isoflurane inhalation was stopped at the time of ketamine/xylazine injection) did not affect the hemodynamic response to another anesthetic.

The time-dependent changes of pO_2 as reported by the OxyLite sensor for different anesthetic regimens are shown in Fig. 2. The ketamine/xylazine mixture caused a rapid drop in both the muscle and tumor pO_2. The decrease in pO_2 was sustained throughout the experiment. In mice anesthetized with fentanyl/droperidol, muscle pO_2 values increased slightly, whereas in the tumor, the response was quite heterogeneous. In 3/9 tumors, pO_2 markedly decreased following fentanyl/droperidol injection, whereas for the others, no change was observed. Pentobarbital (PTB) administration caused a profound reduction in pO_2 in muscle and tumor tissues. At 40 min postinjection, pO_2 declined again, which

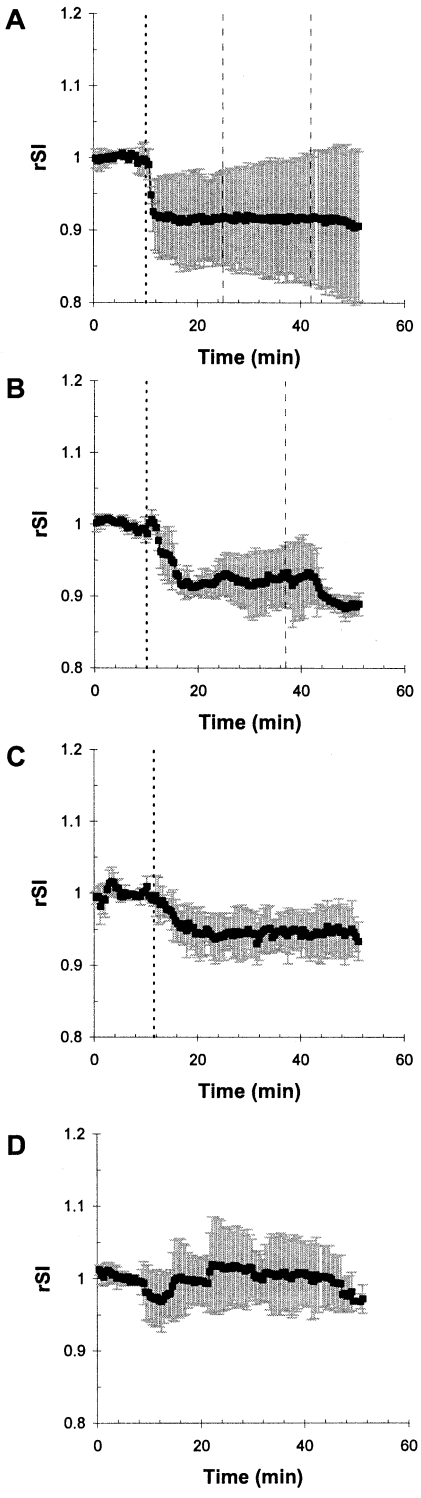

Fig. 1. Relative whole-tumor average change in T_2*w GRE signal intensity on anesthetic administration. (A) ketamine/xylazine, (B) fentanyl/droperidol, (C) pentobarbital, (D) isoflurane. Values are means ± SD.

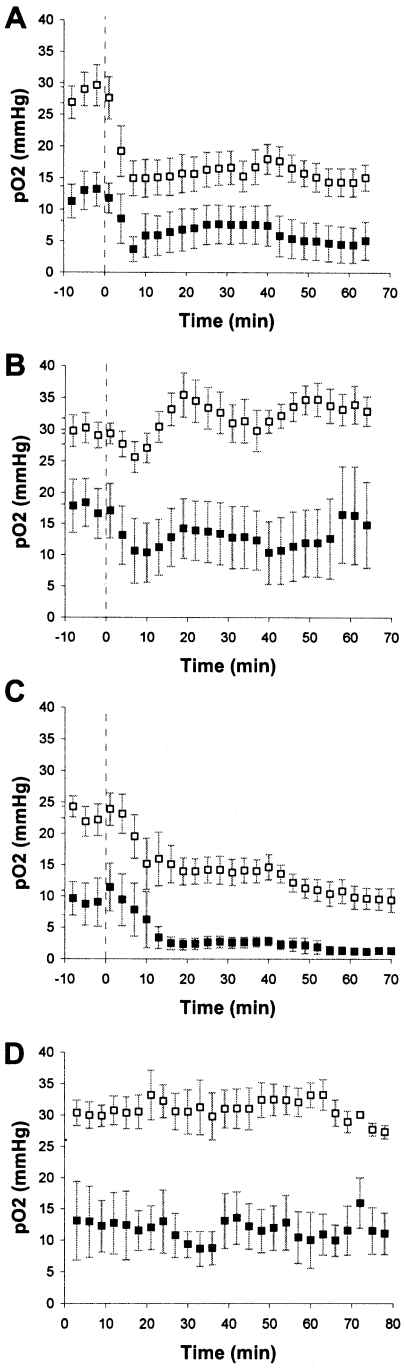

Fig. 2. Time-dependent changes in pO_2 recorded in tumor (■) and in leg muscle (□) on anesthetic administration. (A) ketamine/xylazine, (B) fentanyl/droperidol, (C) pentobarbital, (D) isoflurane. Values are means ± SE.

correlated with the second PTB administration. Under isoflurane anesthesia, muscle pO_2 remained unchanged. Occasionally, transient (lasting 2–8 min), large (up to anoxia) fluctuations in pO_2 were observed in some tumors (see typical record in Fig. 3).

The average pO_2 for muscle and tumor tissues under the four anesthetic regimens are displayed in Fig. 4. Under

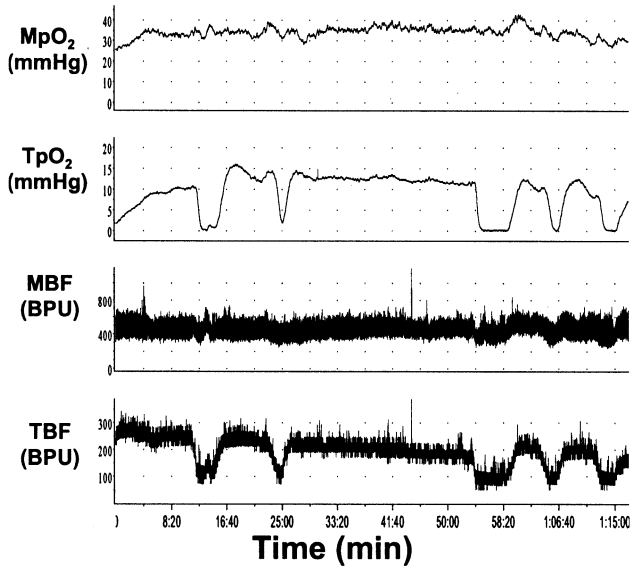

Fig. 3. Typical OxyLite/OxyFlo recordings during isoflurane anesthesia. Large transient fluctuations in tumor blood flow as well in tumor pO_2 are observed. M pO_2: muscle pO_2, T pO_2: tumor pO_2, MBF: muscle blood flow, TBF: tumor blood flow.

isoflurane, pO_2 values were 28.0 ± 0.9 mm Hg and 12.6 ± 1.3 mm Hg for the muscle and TLT tumor, respectively (mean ± SE). Fentanyl/droperidol yielded similar tumor pO_2 values, with even higher pO_2 values in the muscle (32.9 ± 1.2 mm Hg, mean ± SE). Under both ketamine/xylazine and PTB, pO_2 values were significantly lower than under isoflurane. The lowest pO_2 values were observed with PTB anesthesia (12.9 ± 0.9 mm Hg and 2.3 ± 0.4 mm Hg for the muscle and TLT tumor, respectively, mean ± SE).

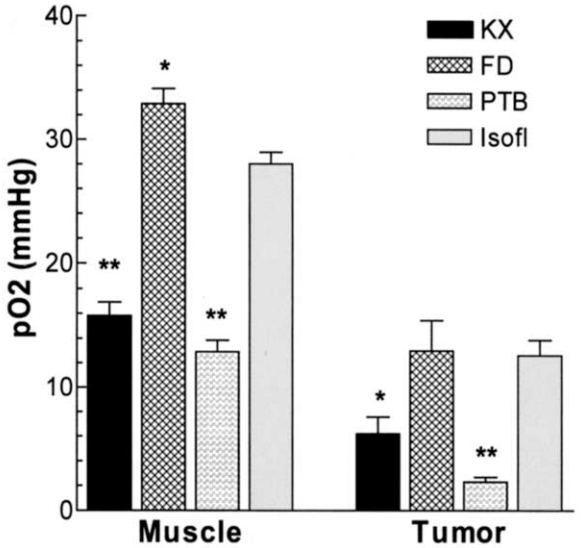

Fig. 4. Muscle and tumor pO_2 under different anesthetic regimens. KX: ketamine/xylazine, FD: fentanyl/droperidol, PTB: pentobarbital, Isofl: isoflurane. Values are means ± SE. *Significantly different from isoflurane ($p < 0.05$). ** Significantly different from isoflurane ($p < 0.01$).

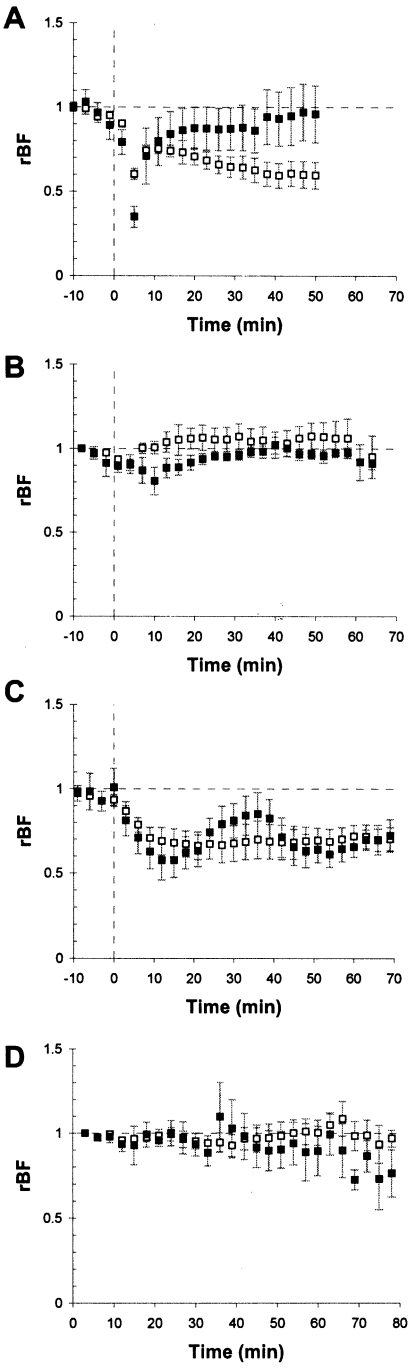

Fig. 5. Time-dependent changes in microvascular red cell flow in tumors (■) and in leg muscle (□) on anesthetic administration (t = 0). (A) ketamine/xylazine, (B) fentanyl/droperidol, (C) pentobarbital, (D) isoflurane. Values are means ± SE.

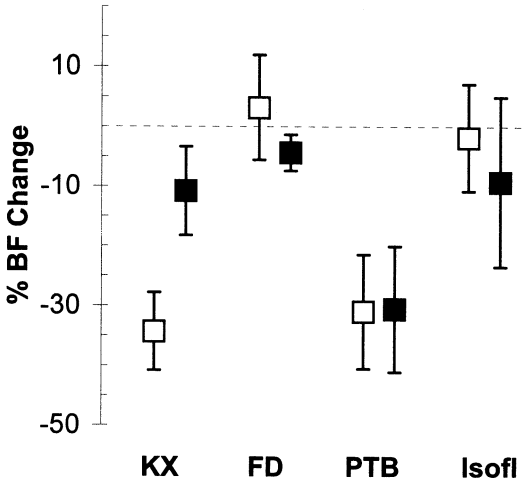

Fig. 6. Change in microvascular red cell flow in tumor (■) and in leg muscle (□) under different anesthetic regimens. KX: ketamine/xylazine, FD: fentanyl/droperidol, PTB: pentobarbital, Isofl: isoflurane. Values are means ± 95% CI.

3.2.3. Microvascular red blood cell flux

Time-dependent relative changes in microvascular red cell flux as reported by the OxyFlo sensor for the different anesthetic regimes are shown in Fig. 5. In ketamine/xylazine-anesthetized mice, a transient profound decrease in blood flow was observed 2–3 min postketamine/xylazine injection in both the muscle and the tumor. The transient fall in tumor perfusion lasted at most 10 min; blood flow values then increased. At the end of the experiment, tumor perfusion was almost back to initial values, whereas muscle perfusion had still lost 40 ± 9% (mean ± SEM) of its initial value. A transient increase in muscle blood flow following repeated administration of ketamine was detected in some mice (not shown). In fentanyl/droperidol anesthetized mice, no change in muscle or tumor perfusion was observed, except during the first 5 min following the fentanyl/droperidol injection. In PTB-anesthetized mice, a marked reduction in muscle blood flow was observed and remained throughout the experiment. Tumor perfusion was also markedly decreased. Under isoflurane anesthesia, no change in muscle blood flow was observed. Transient fluctuations of blood flow were occasionally observed in tumor tissue and these were correlated to fluctuations in pO_2 (see typical record, Fig. 3).

Average changes in relative blood flow for muscle and tumor tissues under the four anesthetic regimens are displayed in Fig. 6. Using ketamine/xylazine and PTB, blood perfusion was significantly decreased. Ketamine/xylazine produced a 34% decrease in muscle blood flow and an 11% decrease in tumor blood flow. PTB produced a 31% decrease in blood flow in both tissues. Muscle blood flow was not significantly affected by fentanly/droperidol, whereas a small decrease (4%) was observed in tumor blood flow.

4. Discussion

In this study, a T_2*w GRE MRI investigation permitted a spatially resolved tumor response to be obtained after administration of commonly used anesthetics. We also measured the effects of the anesthetics on tumor blood flow and tumor pO_2 using the recently commercially available

OxyLite/OxyFlo probe system in order to monitor the time-dependent changes of these parameters simultaneously and continuously. Moreover, a comparison was made with the changes occurring in the skeletal muscle (host tissue).

4.1. Effects of anesthetics on tumor pO_2 and blood flow and comparison with the host tissue (muscle)

The use of anesthetics produced significant alterations in tumor blood perfusion and oxygenation in the TLT tumor model despite the control of normothermia. The most important changes were observed using PTB. PTB caused a profound depression of the pO_2 and blood flow in both the muscle and the tumor, compared with isoflurane. These findings are in agreement with previous studies. Pentobarbital, an oxybarbiturate, has been extensively used in experimental medicine. Previous research has shown that PTB causes myocardial and respiratory depression [22,23], and alterations in microvascular oxygen delivery [24]. Substantial blood flow impairment in the skeletal muscle circulation during PTB anesthesia has been shown previously also; pentobarbital (at 35 or 50 mg/kg) produced a four- to sixfold reduction in skeletal muscle blood flow compared with the awake state in rats [25,26]. Pentobarbital anesthesia is also reported to reduce tumor blood flow and body temperature [9,27]. In our study, pentobarbital yielded very low tumor pO_2 values (2.3 mm Hg) compared with animals under isoflurane anesthesia (12.6 mm Hg). These findings support the observations that pentobarbital anesthesia results in an increase in the hypoxic fraction, and increased radioresistance [9,10,12,13,28].

In mice anesthetized with ketamine/xylazine, a decrease in tumor oxygenation was observed concomitant with the fall occurring in the muscle tissue. As with pentobarbital, these effects could be explained by the variations in blood perfusion. The rapid onset of the fall in blood perfusion compared with pentobarbital could be related to the more rapid anesthetic induction with ketamine/xylazine [29]. Ketamine has been known to cause sympathetically mediated positive inotropic and chronotropic effects, whereas the combination with xylazine, an alpha-2 adrenergic agonist, produces negative chronotropic effects [29]. Yang et al. reported that an ip dose of 150/15 mg/kg ketamine/xylazine reduced heart rate and cardiac output in mice [30]. In DS-carcinosarcoma implanted sc in rats, ketamine/xylazine (50/1 mg/kg) produced a decrease in tumor blood flow associated with a drop in the mean arterial blood pressure [8]. These findings are in agreement with our results. Additionally, the transient increase in muscle blood flow observed in some mice following repeated injections of ketamine, in order to prolong anesthesia, could be explained by its cardiovascular properties. Surprisingly, after the initial drop in blood flow on administration of ketamine/xylazine, there was a trend for perfusion to recover its basal value progressively in tumor tissue, whereas it remained decreased in the muscle tissue. Local metabolic effects of xylazine could explain this different temporal behavior. Xylazine has been shown to cause hyperglycemia [31,32]. Glucose is known to produce a Crabtree effect, i.e., a decrease in oxygen consumption rate, in many tumors without influencing normal tissue [33]. However, an increase in tumor pO_2 would also be expected, which was not obvious in this study [34].

Fentanyl-droperidol is a neurolepanalgesic, a combination of a narcotic analgesic and a tranquilizer. In mice anesthetized with fentanyl/droperidol, oxygenation and blood perfusion remained relatively unchanged for both tumor and muscle, except during the first 5-min postinjection. Our findings are similar to those obtained by Menke and Vaupel where, in rats anesthetized with fentanyl/droperidol (0.075/3.8 mg/kg), no tumor blood flow alterations were detectable, although the mean arterial blood pressure decreased significantly [8]. The pO_2 in some tumors also markedly decreased, while it remained constant (even increased slightly) in the muscle tissue. A redistribution of blood flow or local increase in oxygen consumption could have accounted for these changes.

Isoflurane is a halogenated ether used as an inhalational anesthetic agent. Under isoflurane anesthesia, no overall alterations of tissue perfusion or oxygenation were observed with time. Isoflurane anesthesia is known to yield stable cardiovascular parameters, with retained cardiac output [35]. Zhao et al. also observed no effects of isoflurane (0.8 % v/v) on tumor blood flow in murine RIF-1 tumors grown subcutaneously as compared with conscious mice [16]. However, we showed the presence, individually, of large fluctuations in tumor blood flow and in tumor pO_2 (up to anoxia). These fluctuations may be associated to the tumor acute hypoxia phenomenon. Indeed, spontaneous fluctuations in red cell flux are known to occur in tumors and may even lead to transient hypoxia [36–39]. In this study, we observed these dramatic changes solely under isoflurane anesthesia, suggesting that other anesthetics may obviate such observations. Further investigations need to be undertaken to validate this hypothesis. Yet, previous studies indicated that spontaneous fluctuations in blood flow and pO_2 could also occur under pentobarbital anesthesia [39].

4.2. OxyLite/OxyFlo measurements

The characteristics of the OxyLite/OxyFlo technique we used for monitoring the effect of anesthesia on microvascular blood flow and tissue pO_2 also have to be considered.

First, compared to the polarographic method, the OxyLite system probably overestimates the hypoxic conversion rate. Indeed, OxyLite averages over an area several hundred times larger than the microelectrode (including both vascular and interstitial components), so that it tends to smooth out some of the heterogeneity in tumor response. On the other hand, using a microelectrode, the chance of detecting local pO_2 changes in response to systemic changes is much

more dependent on whether or not the probe is located near a blood vessel [20].

Second, by using fiber-optic probes, continuous measurements of pO_2 and blood flow can be performed. Such methods are highly sensitive to motion so that data need to be acquired under anesthesia. Urano et al. investigated the effect of anesthesia on tumor oxygenation using the OxyLite probe [18]. Surprisingly, continuous increase in pO_2 was observed with pentobarbital and ketamine/xylazine administration at doses similar to ours. This suggested an improvement of the oxygenation status compared to awake mice. However, in this study, probes were inserted 2–3 min after anesthetic administration, so that (a) at this time, measurements may not have reflected basal pO_2 values but rather the early deoxygenated phase following drug administration (e.g., as we observed with ketamine/xylazine anesthesia) and (b) the time for probe stabilization was not taken into account. In contrast, we were able to provide initial blood flow and pO_2 measurements by using isoflurane for which recovery is rapid after the anesthetic gas is turned off. Moreover, we showed that the use of this volatile agent, required to enable a baseline measurement, did not affect pO_2 values reached after administration of an injectable anesthetic agent.

Third, although the OxyLite/OxyFlo method has the advantage of investigating the dynamics of the oxygenation and perfusion status in tissues, only local samples are provided, with the number of measurements limited by the number of channels for the system.

4.3. Effects of anesthetics on BOLD signals in tumors

GRE MRI provided a more global analysis and spatial mapping of these changes than using a local fiber-optic probe. GRE images are sensitive to changes in deoxyhemoglobin concentration, and consequently are sensitive to changes in blood oxygenation and blood volume. They may also be influenced by in-flow effects [5]. This technique has already been used to monitor the effectiveness of treatments designed to alter tumor oxygenation [3–5]. In the present study, a reduction in T_2*w GRE signal intensity was observed in the tumor on administration of pentobarbital, ketamine/xylazine, and fentanyl/droperidol. These results are consistent with the reduction in blood flow and tumor oxygenation observed using the fiber-optic probes. Interestingly, we also reported an opposite response, i.e., an increase in signal intensity, in discrete tumor areas. This finding could be explained by a decrease in blood volume in these tumor regions, which would outweigh the effect of the decrease in blood oxygenation and flow. The decrease in blood volume could have occurred because of a collapse of tumor blood vessels, as a consequence of reduced perfusion pressure in regions with high interstitial blood pressure [5]. Interestingly, in this tumor model, isoflurane had a negligible effect on the evolution of the BOLD signal.

4.4. Extrapolation of these results to other experimental tumor models

The findings in this study relate to one tumor type in one strain of mouse in one growth site. Extrapolation of these results to other experimental tumor models is not straightforward, and these parameters should be checked in any experimental tumor. For example, it has been demonstrated that tumors growing subcutaneously are less sensitive to the effects of anesthesia compared with intramuscular tumors [17]. Indeed, subcutaneous tumors are commonly less well oxygenated than their intramuscular counterparts, likely because they are supplied by a less effective vasculature [17]. Thus, in tumors with intrinsically low pO_2 profiles, it should be expected that the additional worsening of tumor pO_2 on anesthesia would not be detected. Nevertheless, our results could be useful for conducting experiments in radiobiology, MRI, pO_2 measurements in this tumor, as this experimental tumor model is increasingly used to assess the value of pharmacological modulations of the tumor blood flow and pO_2 [3,40–44].

5. Conclusions

Our results emphasize the need for careful monitoring the effect of the anesthesia when trying to identify new therapeutic approaches that are aimed at modulating tumor hemodynamics. The same dramatic effects were found for pentobarbital, ketamine/xylazine, and fentanyl/droperidol using the BOLD-MRI, blood flow, and oxygenation measurements. In the present model, we found that isoflurane had negligible effects on tumor hemodynamics, and, therefore, is optimal when studying the effects of other pharmacological treatments, as it is unlikely to interfere with the measurements obtained by MRI or OxyLite/OxyFlo.

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