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Assessment of the Early Effects of 5,6-dimethylxanthenone-4-acetic acid using Macromolecular Contrast Media Enhanced Magnetic Resonance Imaging: Ectopic versus Orthotopic tumors

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Abstract

Purpose—To investigate the early effects of a vascular disrupting agent (VDA) in ectopic and orthotopic tumors using macromolecular contrast media-enhanced magnetic resonance imaging (MMCM-MRI).

Methods and Materials—MMCM-MRI of ectopic and orthotopic MCA205 murine fibrosarcomas was performed using the intravascular contrast agent, albumin-(Gd-DTPA)₃₅. Change in longitudinal relaxation rate (ΔR_1) was measured 24 hours after treatment with 5,6-dimethylxanthenone-4-acetic acid (DMXAA; 30 mg/kg) and used to compute tumor vascular volume and permeability. Correlative histology and immunohistochemistry was carried out along with measurement of tumor necrosis factor-alpha (TNF- α) and vascular endothelial growth factor (VEGF) levels in whole tumor extracts using the enzyme-linked immunosorbent assay (ELISA).

Results—Orthotopic tumors exhibited higher vascular volume ($P < 0.05$) than ectopic tumors prior to treatment. Twenty-four hours after DMXAA treatment, a significant ($P < 0.0001$) but differential reduction in ΔR_1 (70% in ectopic and 50% in orthotopic tumors) was observed compared to baseline estimates. Consistent with this observation, higher levels of TNF- α , an important mediator of the antivasculature activity of DMXAA were measured in ectopic tumors three hours post treatment compared to orthotopic tumors ($P < 0.05$). Immunohistochemical (CD31) and histological (H&E) sections of ectopic and orthotopic tumors showed a highly tumor selective vascular damage following treatment with the presence of viable surrounding normal tissue.

Conclusions—MMCM-MRI provided early quantitative estimates of change in tumor perfusion following VDA treatment that showed good correlation with cytokine induction. Differences in the response of ectopic and orthotopic tumors highlight the influence of host microenvironment in modulating the activity of VDAs.

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Conflict of Interest: None

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Keywords

VDA; DMXAA; ASA404; MMCM-MRI

Introduction

The process of neovascularization (angiogenesis) is a critical step in malignant progression and a prerequisite for the continued growth of most solid tumors (1). This critical requirement combined with differences in vascular physiology between tumor and normal tissues has led to development of agents that either inhibit angiogenesis or disrupt existing tumor vasculature (1,2). 5,6-dimethylxanthene-4-acetic acid (DMXAA) is a small molecule vascular disrupting agent (VDA) that has successfully completed Phase I-II clinical evaluation in combination with chemotherapy for prostate and lung cancers (3,4). VDAs such as DMXAA are targeted towards the tumor endothelium and result in increased vascular permeability within a few hours after administration followed by blood flow stasis and vascular shutdown (5). As a number of VDAs such as DMXAA progress through clinical trials, it has become evident that due to their varied mechanism of action from cytotoxic anticancer treatments, traditional response assessment criteria alone may not serve as reliable indicators of their pharmacodynamic activity (6,7). Therefore, an essential component of successful clinical evaluation of VDAs is development of non-invasive imaging methodologies to characterize early vascular changes *in situ* following treatment. Among the advanced imaging techniques presently available, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has come to the forefront and is widely being used in clinical trials of VDAs (7). In these studies, physiologic information pertaining to tumor vasculature is obtained by pharmacokinetic modeling of dynamic signal data obtained following administration of a low molecular weight contrast agent containing gadolinium, with gadopentetate dimeglumine (Gd-DTPA; Magnevist®) as the prototype (6,7).

An alternative approach for assessment of tumor vascular function involves the use of macromolecular contrast media (MMCM)-enhanced MRI (8). Initially developed for use in MR angiography, MMCM serve as 'blood-pool' agents and are associated with low first-pass extraction fraction and long circulation times (8,9). These high molecular weight (5-90 kDa) agents do not pass through the normal endothelial barrier and remain in the intravascular space making them ideal for estimating tumor vascular volume and permeability (6,8,9). Comparative studies of macromolecular and low molecular weight contrast agents in preclinical models have highlighted the advantages of using MMCM for characterizing tumor angiogenesis (10). MMCM-MRI based estimates of tumor vascularity have also been successfully correlated with immunohistochemical estimates of microvessel density and histological tumor grade (11). The overall goal of the present study was to utilize MMCM-MRI to examine the early tumor vascular response to DMXAA.

It is now well recognized that the host microenvironment strongly influences tumor angiogenesis and response to therapy (12,13). While the preclinical activity of DMXAA against subcutaneous tumors has been extensively studied, the antivascular effects of DMXAA on tumors of the same histological type implanted at ectopic and orthotopic locations has not been investigated. In the present study, to examine the influence of the tissue microenvironment on tumor vascular response to DMXAA, studies were carried out using murine fibrosarcomas implanted at ectopic (subcutaneous) and orthotopic (intramuscular) tissue implantation sites. In a previous study, using a subcutaneous murine tumor model, we have shown that DMXAA results in a marked increase in tumor vascular permeability four hours after treatment and subsequently leads to hemorrhaging and reduction in tumor perfusion at twenty four hours (14). Therefore, in this study, we chose to investigate the vascular response of ectopic and orthotopic murine tumors to DMXAA at the 24 hour time point after a single injection of

DMXAA. Quantitative estimates of vascular volume and permeability were calculated from change in longitudinal relaxation rate ($\Delta R1$) following administration of albumin-(Gd-DTPA)₃₅, a well-characterized macromolecular MR contrast agent that consists of Gd-DTPA chelates covalently conjugated to human serum albumin (15). Correlative histopathologic examination along with measurement of intratumoral levels of tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF), important mediators of the antivasculature activity of DMXAA, were performed.

Materials and Methods

Tumor model

Female C57Bl6 mice (Jackson Laboratories, Bar Harbor, ME) were fed food and water *ad libitum* and housed in microisolator cages under ambient light. Methylchoanthrene-induced fibrosarcomas (MCA205) were established by injecting 3×10^5 cells either subcutaneously (ectopic) or in the leg muscle (orthotopic) of six-to-eight week old mice under transient anesthesia, in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC). Experimental studies were carried out on tumor-bearing mice approximately 15-18 days post implantation when the mean tumor volumes ranged from ~ 100 - 175 mm^3 .

Chemicals

DMXAA (*courtesy of Gordon Rewcastle, University of Auckland, New Zealand*) was freshly prepared in 5% sodium bicarbonate prior to intraperitoneal (i.p.) injection at a dose of 30 mg/kg. Albumin-(Gd-DTPA)₃₅ (*courtesy of Dr. Robert Brasch*) was obtained from the Contrast Media Laboratory, University of California at San Francisco, San Francisco, CA.

MMCM-MRI

Studies were carried out in a 4.7T/33 cm horizontal bore magnet (GE NMR Instruments, Fremont, CA) incorporating AVANCE digital electronics (Bruker Biospec with ParaVision 3.0.2; Bruker Biospin, Billerica, MA). Mice were anesthetized using isoflurane (Abbott Laboratories, Chicago, IL), secured in a form-fitted MR-compatible mouse sled (Dazai Research Instruments, Toronto, Canada) and positioned in the scanner. Animals were kept warm during image acquisition using a water bath maintained at 37°C or an air heater system (SA Instruments Inc., Stony Brook, NY) connected to a thermocouple embedded within the sled that provided feedback for automated temperature control. Multislice relaxation rate ($R1 = 1/T_1$) maps were obtained using saturation recovery, fast spin echo (FSE) scans with variable repetition times before and after contrast agent administration as described previously (16). Following baseline acquisitions, albumin-(Gd-DTPA)₃₅ was administered at a dose of 0.1 mmol/kg as a bolus *via* tail-vein injection and post contrast images were acquired over ~ 50 minutes. Axial images were collected from at least 2-3 slices through the whole tumor. Kidneys were sampled to estimate the concentration of contrast agent in the blood.

Image processing

Region of interest (ROI) selection and MR data analysis were carried out using Analyze PC (Version 7.0, AnalyzeDirect, Overland Park, KS) and MATLAB (Version 7.0; Mathworks Inc., Natick, MA). The relaxation rate $R1$ and the maximal signal intensity $S_{\max}$ were calculated following subtraction of background noise using the following equation

$$S_{\text{TR}} = S_{\max} (1 - e^{-(R1 \cdot \text{TR})})$$

where S_{TR} is the signal intensity obtained at each TR time. The change in longitudinal relaxation rate of different tissues [for example, tumor, kidneys etc; $\Delta R1(t)$] after contrast agent injection was calculated according to the following equation.

$$\Delta R1(t) = 1/T1(t)_{post} - 1/T1(t)_{pre}$$

where $T1(t)_{pre}$ and $T1(t)_{post}$ represent the longitudinal relaxation times of the tissue before and after contrast agent injection, respectively. Average baseline R1 values of the three precontrast scans was subtracted from the postcontrast R1 values from each of the 4 post contrast scans to obtain the change in longitudinal relaxation rate, $\Delta R1$ over time. The slope of $\Delta R1$ versus time was used to determine vascular permeability and the intercept of the line at time zero was used to estimate tumor vascular volume (10,16). R1 maps were generated on a pixel-by-pixel basis using MATLAB.

Data analysis

Comparative analysis of vascular differences between ectopic and orthotopic tumors was carried out using volume-matched data sets [mean $\pm$ standard error for ectopic (101 ± 31 mm³) and orthotopic (175 ± 34 mm³) tumors, $P > 0.05$]. Vascular response to DMXAA was assessed using paired data sets obtained for 4 mice bearing ectopic tumors before (*baseline*) and 24 hours post DMXAA (*24h post*). For orthotopic tumors, a total of 6 tumor-bearing mice were scanned before and 24h after DMXAA treatment. However, data from one animal (m1) at *baseline* was discarded due to unacceptable motion and was replaced with a separate data set from another animal (m6) bearing a volume-matched control tumor. Data from another animal (m4) was discarded at the 24 hours post time point due to bad injection. Data analysis of orthotopic tumors was therefore carried out using 6 tumors for *baseline* and 5 tumors for *24h post* time points.

Immunohistochemistry and histology

Tumors were harvested from untreated controls and DMXAA-treated animals and placed in Tris-buffered zinc fixative for histology and immunohistochemistry. Immunostaining for the pan endothelial cell adhesion molecule, CD31 (PECAM) was performed as described previously (16). Slides were counterstained with Harris hematoxylin (Poly Scientific, Bayshore, NY).

Enzyme-linked immunosorbent assay

Determination of protein levels of TNF- α and VEGF was performed using enzyme-linked immunosorbent assay (ELISA) on tissue samples isolated from a separate cohort of 3-4 mice per group as described previously (14).

Statistical Analysis

All measured values are reported as the mean $\pm$ standard error of the mean. The two-tailed *t*-test was used for comparing data between control and treatment groups. *P* values less than 0.05 were considered statistically significant. All statistical calculations and analyses were performed using GraphPad Prism (Version. 5.00 for Windows, GraphPad Software, San Diego, CA).

Results

To examine the influence of the tissue microenvironment on tumor vascularity *in vivo*, MMCM-enhanced MRI was performed on ectopic (n=4) and orthotopic (n=6) fibrosarcomas. As shown in Fig. 1A, R1 maps of ectopic and orthotopic MCA tumors showed differences in

enhancement between orthotopic and ectopic tumors. Orthotopic MCA tumors appeared as lobular structures within the leg muscle and showed distinct enhancement on the tumor periphery (*lower panel*). In comparison, ectopic tumors showed minimal enhancement post contrast (*upper panel*). The change in tumor R1 ($\Delta R1_t$) following albumin-(Gd-DTPA)₃₅ injection was quantitated and normalized to $\Delta R1$ values of blood ($\Delta R1_b$) as an indirect measure of blood flow. As shown in Fig. 1B, orthotopic MCA tumors showed a greater increase in $\Delta R1$ values (0.3046 ± 0.058) than ectopic MCA tumors (0.0966 ± 0.031) indicative of increased perfusion ($P < 0.001$).

To further investigate vascular differences between ectopic and orthotopic MCA tumors prior to DMXAA treatment, linear regression analysis of the temporal change in $\Delta R1$ was performed to calculate the slope and y-intercept value at time zero. The slope represents the permeability of the tumor vessels to albumin-(Gd-DTPA)₃₅ and the y-intercept provides a measure of tumor vascular volume (VV) (10,16). Orthotopic tumors exhibited ~3-fold greater VV (0.203 ± 0.031 , Fig. 2B control) than ectopic tumors (0.073 ± 0.008 , Fig. 2A, control). Ectopic MCA tumors (Fig. 2A, control) showed an increase in $\Delta R1$ values over the 50 minute period following contrast agent administration (slope, 0.00152 ± 0.0002 , $r^2 = 0.9435$). In comparison, orthotopic tumors (Fig 2B, control) showed minimal accumulation of contrast agent over time (slope 0.00069 ± 0.0008 , $r^2 = 0.2498$).

Twenty four hours after DMXAA treatment, MMCM-MRI revealed a significant reduction in VV ($P < 0.001$) in both ectopic and orthotopic tumors following DMXAA treatment. However, the extent of reduction in VV in response to DMXAA treatment varied between ectopic and orthotopic tumors. Ectopic MCA tumors (Fig. 2A) showed ~70% decrease in VV following DMXAA treatment (0.022 ± 0.020 , $n=4$) compared to baseline values. In comparison, orthotopic MCA tumors exhibited only ~50% reduction in VV (0.109 ± 0.005 , $n=5$) following DMXAA treatment (Fig. 2B). No statistically significant difference was observed in $\Delta R1$ ($P > 0.1$) values of kidneys (calculated as surrogate measure of contrast agent concentration in the blood) between animals in control and treatment groups for both ectopic and orthotopic tumors (Fig. 2C).

To visualize the heterogeneity in the vascular response of ectopic and orthotopic tumors to DMXAA, R1 maps were generated on a pixel-by-pixel basis immediately post contrast (*baseline*) and 24 hours post treatment (*24h post*). As shown in Figure 3, 24 hours following DMXAA treatment, R1 maps of ectopic MCA tumors exhibited markedly bright (hyperintense) regions within the tumor (*ROI*) indicative of marked vascular damage. In comparison, R1 maps of orthotopic MCA tumors showed areas of moderate change within the tumor (*ROI*) 24 hours following treatment compared to baseline R1 maps.

Vascular status was also assessed by immunostaining of tumor sections for the endothelial cell marker, CD31. Hematoxylin and eosin (H&E) staining was used to assess tissue necrosis. Both ectopic and orthotopic tumor sections showed evidence of vascular damage 24 hours following DMXAA treatment (Fig. 4). Consistent with previous observations, CD31/H & E staining revealed extensive areas of hemorrhagic necrosis devoid of CD31 staining along with viable tumor cells and CD31+ blood vessels in the tumor rim (Fig. 4, *upper and lower panels*). Interestingly, CD31 immunostained sections of orthotopic MCA tumors showed a highly selective vascular response to DMXAA with intact vasculature visible in the neighboring muscle tissue (Fig. 4 *lower panel, M*). Analysis of $\Delta R1$ values of muscle tissue (data not shown) were consistent with this observation and showed no statistically significant difference between control and treatment groups ($P > 0.5$).

Finally, we determined if the differential vascular response to DMXAA between ectopic and orthotopic MCA tumors correlated with intratumoral levels of TNF- α , a principal cytokine

involved in antivascular activity of DMXAA (17,18). Differences in intratumoral VEGF levels were also analyzed. As shown in Fig. 5A, untreated control MCA tumors established at ectopic and orthotopic tissue sites showed extremely low levels of TNF- α , (0.33 ± 0.03) and (3.5 ± 1.7), respectively. Three hours post DMXAA treatment, ectopic MCA tumors (638.5 ± 169.2 pg/ml/40 μ g protein, $P < 0.01$ vs controls) showed ~6-fold greater ($P < 0.05$) induction of TNF- α compared to orthotopic MCA tumors (106.66 ± 8.76 pg/ml, $P < 0.001$ vs controls). No statistically significant difference in intratumoral levels of VEGF were observed between untreated ectopic and orthotopic MCA tumors ($P > 0.05$). However, higher levels of VEGF were seen in orthotopic tumors ($P < 0.05$) than ectopic tumors following DMXAA treatment (Fig. 5B).

Discussion

The host microenvironment is critically involved in tumor angiogenesis through a complex network of interactions between tumor cells, endothelial cells and host cells (12,13). It is therefore important to evaluate and interpret the preclinical activity of VDAs within the context of the tumor type and its microenvironment. In the present study, non-invasive MMCM-MRI was utilized to investigate the influence of the host microenvironment on tumor angiogenesis and response to DMXAA. The results demonstrate the usefulness of MMCM-MRI in characterizing vascular differences between ectopic and orthotopic tumors and provide evidence for the early vascular disruptive effects of DMXAA *in vivo*. Orthotopic tumors exhibited increased vascular volume compared to ectopic tumors (Figs 1 and 2). While the effect of implantation site on tumor vascular characteristics is likely to vary depending on the model system evaluated, similar findings have been previously reported (19). Using MMCM-MRI, Kim *et al.*, have shown that the blood volume of orthotopic colon tumors was higher than ectopic tumors (19). In contrast, Zechmann and colleagues have shown that experimental hormone-sensitive orthotopic prostate tumors exhibit decreased perfusion compared to subcutaneous tumors (20).

The early effects of DMXAA observed in preclinical tumor models include changes in vascular permeability leading to extravasation of proteins, increased viscosity, blood flow stasis and eventual vascular collapse and tissue necrosis (5,17). Several studies by us and others have reported potent vascular disruptive activity of DMXAA across a range of subcutaneous animal and human tumor models (16-18). Recently, the antitumor activity of DMXAA against chemically-induced mammary tumors in rats has also been investigated (21). To the best of our knowledge, this is the first study to investigate the antivascular activity of DMXAA using the same histological tumor type established at ectopic and orthotopic locations.

The initial impetus for the development of DMXAA was its ability to induce high levels of TNF- α *in situ* (18). In our study, MMCM-MRI results revealed a differential vascular response between ectopic and orthotopic tumors to DMXAA, with ectopic tumors exhibiting a greater reduction in vascular volume than orthotopic tumors (Fig. 2). Consistent with this observation, analysis of TNF- α levels 3 hours post treatment showed increased TNF- α levels in ectopic tumors compared to orthotopic tumors. The effects of TNF- α on endothelial integrity and permeability have been previously demonstrated (22,23). Using TNF gene knockout^{-/-} mice, it has been shown that tumor cells synthesize TNF mRNA and protein following DMXAA treatment (24). Marked attenuation of antitumor activity has also been observed following DMXAA treatment in murine colon 38 tumors grown in TNF receptor^{-/-} mice (25). In the same study, it was also shown that TNF receptor^{-/-} mice tolerated higher levels of DMXAA than wild-type counterparts implicating TNF in the host toxicity and antitumor activity of DMXAA (25). Additionally, studies carried out by us and others have reported the onset of endothelial apoptosis as early as 30 minutes following drug administration suggestive of direct drug effects on the endothelium (14,26). It is now believed that the antivascular effects of DMXAA are a

consequence of both direct drug effects on tumor endothelial cells and indirect effects mediated by cytokines and growth factors (5,14,26). In a recent study, good correlation was observed between plasma levels of the serotonin metabolite, 5-hydroxy indole acetic acid (5-HIAA) and the onset of tumor vascular damage by DMXAA (27). Although the precise mechanism of DMXAA-induced vascular disruption is not clear, recent studies have identified targets in NF κ B and MAPK biochemical pathways (28,29).

It is now widely acknowledged that due to their varied mechanism of action, clinical evaluation of VDAs will require an alternative approach than measures of tumor morphology or size (5-7). In this regard, non-invasive imaging techniques such as MRI can be successfully used to detect early vascular changes a few days after treatment. Imaging-based parameters of vascular function could also potentially serve as markers of antivascular activity in clinical trials (7). Indeed, Phase I trials of VDAs such as DMXAA and combretastatin-A-4-phosphate (CA4P) have included DCE-MRI investigations to determine evidence of antivascular activity in patients with promising results (30,31). Interpretation of DCE-MRI data is based on pharmacokinetic modeling of intravascular and extravascular distribution of Gd-DTPA to obtain parameters such as K_{trans} and area under the curve (AUC). However, these DCE-MRI measures represent a combination of tissue parameters such as perfusion, permeability and vessel surface area. This becomes particularly relevant as VDAs such as DMXAA result in changes in vascular permeability within a few hours after treatment followed by a marked reduction in blood flow (5,14). Enhanced permeability following DMXAA treatment would potentially increase the extravascular distribution volume of Gd-DTPA while reduced perfusion would decrease vessel surface area and extravascular distribution, complicating data interpretation and potentially leading to confounding results (30). Such an observation was reported by McPhail et al., in which no change in DCE-MRI parameters were observed following DMXAA treatment in rat tumors despite a marked increase in 5-hydroxyindoleacetic acid (5-HIAA) (27). The use of freely diffusible low molecular weight contrast agents has also contributed to inconsistent observations in clinical trials. In the Phase I trial of DMXAA, changes in DCE-MRI parameters, gradient, enhancement and area under the gadolinium concentration curve were used as indirect measures of antivascular activity (30). In spite of the observed reduction in these parameters following treatment, a dose response relationship was not observed. While tumor and patient heterogeneity could have contributed to this effect, the authors acknowledge the limitations associated with the use of pharmacokinetic DCE-MRI parameters that rely on signal intensity change (30). The relaxation rate of tissues rather than signal enhancement is proportional to the contrast agent concentration (32). Therefore, kinetic analysis of the change in the relaxation rate of tissues following administration of a macromolecular contrast agent is likely to provide a better measure of tissue vascular volume. Using this approach, several preclinical studies have successfully utilized MMCM-MRI to determine changes in vascular volume and permeability following treatment (8-11). Preda *et al* have utilized MMCM-MRI to characterize changes in vascular permeability in rat mammary tumors following treatment with the humanized monoclonal VEGF antibody, Bevacizumab (33). While clinical translation of MMCM has been hindered by safety concerns related to immunogenicity and gadolinium accumulation in normal tissues, recent results using MMCM have been encouraging (8,9,34). Human studies using ultrasmall paramagnetic iron oxide (USPIO) particles and intermediate size agents like Gadomer-17 have demonstrated good safety profiles and signal-to-noise ratios (9,34). Future clinical approval of some of these agents should allow translation of MMCM-MRI to monitor the pharmacodynamic activity of VDAs in patients.

Finally, while the results of our study demonstrate the potent antivascular activity of DMXAA, only a single dose of DMXAA was evaluated and direct correlation of MMCM-MRI-based early vascular changes with long-term treatment outcome was not performed. Such a study design using a large cohort of animals and multiple DMXAA doses to determine the predictive

ability of MMCM-MRI parameters to serve as potential ‘biomarkers’ of biological activity and long-term outcome is currently being planned.

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Figure 1.

(A) Axial T1-weighted MR images and R1 maps of ectopic and orthotopic MCA tumors following administration of albumin-(Gd-DTPA)₃₅. (B) Change in longitudinal relaxation rate ($\Delta R1_t$) in the tumor following contrast agent administration normalized to change in relaxation rate of kidneys ($\Delta R1_b$). Kidneys were used as a surrogate measure of contrast agent concentration in the blood. * $P < 0.05$ (Ectopic $n=4$, Orthotopic $n=6$).

Figure 2.

MMCM-MRI based estimates of tumor vascular response to DMXAA. Change in tumor $\Delta R1$ of control (*baseline*) and DMXAA-treated (*24h post treatment*) ectopic (A; n=4 per group) and orthotopic (B; n = 6 controls, 5 DMXAA) fibrosarcomas. (C) $\Delta R1$ values of kidneys (blood) of mice from ectopic and orthotopic control and treatment groups were calculated to account for variability in contrast agent injection ($P>0.1$).

Figure 3.

Axial proton density weighted (*proton*; TE/TR =25/6000), calculated R1 maps and enlarged regions-of-interest (*ROI*; tumor) of mice bearing ectopic and orthotopic fibrosarcomas before (*baseline*) and 24 hours post DMXAA (*24h post*). Enlarged ROIs highlight the differential vascular response of ectopic and orthotopic tumors to DMXAA.

Figure 4.

Photomicrographs of CD31 and H&E stained tumor sections of control and DMXAA-treated ectopic and orthotopic fibrosarcomas (10×magnification, n=3 mice per group). Central regions devoid of CD31 staining were visible along with viable tumor cells and CD31+ blood vessels in the tumor rim. Vascular response to DMXAA was selective to the tumor with intact vasculature visible in the neighboring muscle (M) tissue (*lower panel*).

Figure 5.

Intratumoral levels of tumor necrosis factor-alpha (A) and vascular endothelial growth factor (B) in untreated controls and DMXAA-treated tumors 3 hours post treatment measured using ELISA (n=3-4 mice per group). Consistent with the MRI data, ectopic tumors showed increased TNF induction compared to orthotopic tumors following DMXAA treatment. *P<0.05, **P<0.01, ***P<0.001.