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CONTRAST ENHANCED MRI ASSESSMENT OF TUMOR BLOOD VOLUME AFTER APPLICATION OF ELECTRIC PULSES

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ABSTRACT

The effect of application of short, intense electric pulses on tumor blood volume was investigated using albumin–$(Gd\text{-}DTPA)_{30}$ contrast-enhanced magnetic resonance imaging (MRI). One of paired SA-1 fibrosarcoma tumors implanted in each flank of A/J mice was treated with electric pulses. MRI was performed dynamically before and after intravenous administration of albumin–$(Gd\text{-}DTPA)_{30}$ (0.02 mmol Gd/kg), and fractional tumor blood volume was estimated. MRI images of tumors exposed to electric pulses showed no enhancement at 30 min after injection of albumin–$(Gd\text{-}DTPA)_{30}$. However, marked enhancement was observed in paired tumors of the same mice that were not exposed to electric pulses. A significant difference in blood volume was observed between nontreated tumors and tumors treated with electric pulses. Application of electric pulses to the tumors significantly reduced blood volume in the tumors. Therefore, through a reduction in tumor blood volume, electric pulses may, besides producing

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electroporation of cells, exert antitumor effectiveness by entrapping drugs within the tumors.

INTRODUCTION

Contrast enhancement of malignant neoplasms, using small or larger molecular gadolinium chelates, is well documented (1–5). Gd-DTPA equilibrates rapidly between intracellular and extracellular spaces, and because of its small molecular size cannot serve as a measure for relative blood volume (1). The distribution volume of macromolecular contrast media, represented by albumin–$(Gd\text{-}DTPA)_{30}$, which has a plasma half life of approximately 3 h, closely approximates the blood volume (6,7). Albumin–$(Gd\text{-}DTPA)_{30}$ produces nearly constant enhancement of blood and normal tissues for 60 min or longer after injection (7,8). The enhancement curve in tumors indicates disrupted endothelium, yet this curve closely approximates relative blood volume in the first 5 min after administration of albumin–$(Gd\text{-}DTPA)_{30}$ (5,9).

Many therapies and drugs have been shown to induce perturbation of tumor blood flow. Some of the modalities that do this are hyperthermia (10), photodynamic therapy (11), high-energy shock waves (12), cytokines such as tumor necrosis factor-α (TNF-α) (13) and interleukin-1 (IL-1) (14), and drugs such as hydralazine, serotonin, flavonic acetic acid, and *Vinca* alkaloids (15,16). All of these treatment modalities can also be exploited to improve therapeutic outcome in combination with other treatment modalities or bioreductive drugs that take advantage of tumor hypoxia.

Biomedical applications of electric pulses have until now been used predominantly either for insertion of drugs, genes, or dyes into cells *in vitro*, or for drug and gene delivery into cells and tissues *in vivo* (17). Electrochemotherapy utilizes this approach to potentiate the antitumor effectiveness of chemotherapeutic drugs such as bleomycin and cisplatin by application of electric pulses to tumors (18–21).

This study was undertaken to determine the effects of electric pulses on tumor blood flow, as measured with contrast-enhanced magnetic resonance imaging (MRI) using a macromolecular contrast agent. This may have implications in demonstrating that besides electroporation, electric pulses may have a blood-volume-modifying effect, which could contribute to the antitumor effectiveness of electrochemotherapy *in vivo* by entrapment of drugs in the tumor.

MATERIALS AND METHODS

Animals and Tumor Model

In the experiments, an inbred strain of A/J mice was used, purchased from Rudjer Bošković Institute (Zagreb, Croatia). They were maintained at constant room temperature (24°C) with a natural day/night light cycle in a conventional animal colony. Before the experiments, the mice were subjected to an adaptation period of at least 10 days. Mice in good condition, without fungal or other infections and 12–14 weeks of age, were included in the experiments.

In the study, the fibrosarcoma SA-1 tumor model (Jackson Laboratory, Bar Harbor, ME), syngeneic to A/J mice, was used. Tumor cells were obtained from the ascitic form of the tumors in mice, and were serially transplanted every 7 days. Paired solid subcutaneous SA-1 fibrosarcomas, implanted in each flank of A/J mice, were initiated

by an injectiion of 5×10^5 SA-1 cells in 0.1 ml 0.9% NaCl solution. The viability of the cells was over 95% as determined by the trypan blue dye exclusion test. Six days after transplantation, when the tumors reached approximately 40 mm^3 in volume, mice were subjected to a specific experimental protocol.

MRI Contrast Medium

Albumin–$(Gd\text{-}DTPA)_{30}$ (synthesized by Jeffry Mann, Contrast Media Laboratory, Department of Radiology, University of San Francisco, San Francisco, CA), a prototype intravascular-blood-pool contrast agent for MRI, was used. This compound has been well characterized as a blood-pool contrast agent (6,7,22).

Application of Electric Pulses and MRI

Electric pulses were delivered by two flat, parallel stainless-steel electrodes 8 mm apart (two stainless-steel strips, length 35 mm, width 7 mm, with rounded corners). The electrodes were placed percutaneously at the opposite margins of the tumor. Good contact between the electrodes and the skin was assured by means of conductive gel (Parker Laboratories, Inc., NY). Eight square-wave, high-voltage, direct-current (DC) electric pulses with amplitude 1040 V, pulse width 100 μs, and repetition frequency 1 Hz were generated by a Jouan GHT 1287 electropulsator (Jouan, France). Treatment with electric pulses was performed without anesthesia and was well tolerated by the mice.

Thirty minutes after application of electric pulses to one tumor on the right flank, albumin–$(Gd\text{-}DTPA)_{30}$ (0.02 mmol Gd/kg) was administered via a catheter into the lateral tail vein of the anesthetized mouse. Anesthesia was induced by intraperitoneal injection of 100 mg/kg of ketamine (Ketanest; Parke-Davis, Berlin, Germany), 10 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany), and 0.1 mg/kg of atropine. Before administration of the contrast agent, a nonenhanced set of images was obtained. Subsequently, serial dynamic MRI images were made at 2-min intervals for 30 min to generate 10 postcontrast image sets for computer analysis.

MRI was performed on a Bruker Biospec system (Bruker Medizin Technik GmbH, Ettlingen, Germany), operating at 2.35 T. The system is equiped with Oxford gradient coils (inner diameter: 22.5 cm) and a saddle radio-frequency (RF) coil. For imaging mouse tumors, T_1-weighted, dynamic, multislice spin-echo images were obtained with the following parameter settings: TR = 200 ms, TE = 10 ms, flip angle = 90°, matrix = 256 × 256, slice thickness = 2 mm, FOV = 6 cm, and acquisition time 104 s.

Data Analysis

To obtain a measure of MR signal intensity proportional to tissue albumin concentration C_T, a $\Delta SI(t)$ map was constructed by subtracting the signal intensity before contrast agent was administered from the values after contrast agent was administered on a pixel-by-pixel basis (23–26). MRI provides a dynamic estimate of tissue $C_T(t)$, which is equal to the sum of contrast agent concentration in the tissue vascular space $C_{T\text{-}B}(t)$ plus the concentration in the extravascular interstitial space $C_{T\text{-}E}(t)$ when tissue-compartment concentrations are expressed in terms of mass of contrast medium per unit volume of whole tissue such that:

$$C_T(t) = C_{T\text{-}B}(t) + C_{T\text{-}E}(t) \quad (1)$$

Tissue blood volume is obtained by linear regression of Equation 1 at t = 0.

The method for estimating tissue blood volume has been reported in detail (23, 24). Using the same method, blood volume was calculated on a pixel-by-pixel basis (26).

RESULTS

Dynamic images showed little or no enhancement of the tumors 30 min after exposure to 8 electric pulses. However, marked enhancement was observed in paired tumors in the same mice that were not exposed to electric pulses. Representative images in Figure 1 show the temporally related increase in signal intensity in the tumor that was not exposed to electric pulses, due to increasing accumulation of albumin–$(Gd\text{-}DTPA)_{30}$. Blood-volume maps revealed a difference in blood volume between tumors that were exposed to electric pulses and those that were not exposed. Region-of-interest (ROI) analysis in the tumor rims, which included at least 50 pixels, showed changes in blood volume in the tumors that were not exposed to electric pulses (blood volume = 20 ± 8%) compared with the tumors that were exposed (blood volume = 0 ± 3%).

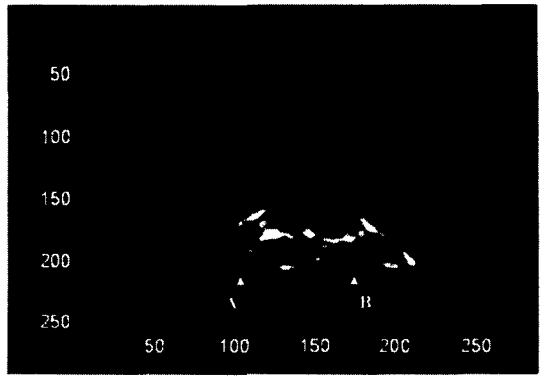
DISCUSSION

This study shows that application of electric pulses significantly reduces the blood volume of tumors. Because of this reduction in tumor blood volume, electric pulses may, besides producing electroporation of cells, exert an antitumor action by the entrapment of drugs within the tumors.

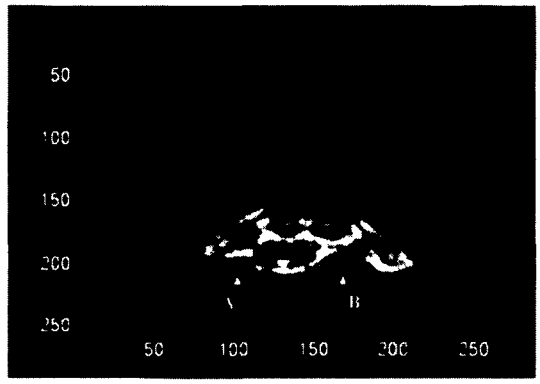
Electrochemotherapy is an antitumor treatment that utilizes electric pulses to permeabilize the plasma membrane and thus enables the access of drugs into tumor cells in order to exert cytotoxocity (17). Drug-delivery properties of electric pulses have been demonstrated for several drugs, mainly those that are non- or poorly permeant. Among these drugs, it has been proven that the application of electric pulses potentiated by several fold the cytotoxocity of bleomycin and cisplatin. This therapy has also proven very effective *in vivo*, as demonstrated in animal tumors and in the treatment of human cutaneous malignancies (18–21,27–29).

It has often been suggested that electric pulses may have blood modifying effects. This possibility was supported by the observation that the platinum (Pt) content in tumors was increased and prolonged after the application of electric pulses (30). This observation may have been due to entrapment of the chemotherapeutic drug cisplatin in the electroporated cells and/or entrapment of the drug within the tumors, owing to perturbation of tumor blood flow. If tumors exposed to electric pulses have a reduced blood flow, this could result in prolonged pharmacokinetic exposure of the tumor cells to a chemotherapeutic agent.

In the present study it was demonstrated that rapid and profound tumor blood-flow shutdown was obtained after the application of 8 electric pulses with an amplitude of 1040 V. Such amplitudes are usually used in electrochemotherapy of tumors *in vivo*. Therefore, in electrochemotherapy, besides electroporation of the cells in the tumor, reduction of tumor blood flow also occurs, within 30 min after the application of pulses. Depending on the duration of this blood-flow-modifying effect of electric pulses, it may contribute significantly to the antitumor effectiveness of electrochemotherapy. Further



(A)



(B)

FIGURE 1. (A) Precontrast image and (B) image at 2 min after injection of contrast medium. Paired tumors are indicated with arrows; A indicates nontreated tumor and B indicates tumor treated with electric pulses.

studies are needed to characterize the time course of changes in tumor blood flow and their dependence on the amplitude and number of electric pulses applied.

Besides reflecting changes in relative blood volume, macromolecular contrast agents offer a means for measuring changes in the permeability of tumor vessels. The study of microvascular permeability may provide additional insight into the effects of electrochemotherapy on tumors.

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