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Combined molecular MRI and immuno-spin-trapping for in vivo detection of free radicals in orthotopic mouse GL261 gliomas

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ABSTRACT

Free radicals play a major role in gliomas. By combining immuno-spin-trapping (IST) and molecular magnetic resonance imaging (mMRI), in vivo levels of free radicals were detected within mice bearing orthotopic GL261 gliomas. The nitrone spin trap DMPO (5,5-dimethyl pyrroline *N*-oxide) was administered prior to injection of an anti-DMPO probe (anti-DMPO antibody covalently bound to a bovine serum albumin (BSA)–Gd (gadolinium)-DTPA (diethylene triamine penta acetic acid)–biotin MRI contrast agent) to trap tumor-associated free radicals. mMRI detected the presence of anti-DMPO adducts by either a significant sustained increase ($p < 0.001$) in MR signal intensity or a significant decrease ($p < 0.001$) in T_1 relaxation, measured as $\%T_1$ change. In vitro assessment of the anti-DMPO probe indicated a significant decrease ($p < 0.0001$) in T_1 relaxation in GL261 cells that were oxidatively stressed with hydrogen peroxide, compared to controls. The biotin moiety of the anti-DMPO probe was targeted with fluorescently-labeled streptavidin to locate the anti-DMPO probe in excised brain tissues. As a negative control a non-specific IgG antibody covalently bound to the albumin–Gd-DTPA–biotin construct was used. DMPO adducts were also confirmed in tumor tissue from animals administered DMPO, compared to non-tumor brain tissue. GL261 gliomas were found to have significantly increased malondialdehyde (MDA) protein adducts ($p < 0.001$) and 3-nitrotyrosine (3-NT) ($p < 0.05$) compared to normal mouse brain tissue, indicating increased oxidized lipids and proteins, respectively. Co-localization of the anti-DMPO probe with either 3-NT or 4-hydroxynonenal was also observed. This is the first report regarding the detection of in vivo levels of free radicals from a glioma model.

1. Introduction

Reactive oxygen (and nitrogen) species (ROS/RNS) generated from oxidative stress play a crucial role in cancers such as gliomas, either as modulators of signal transduction or as a causal agent of tissue injury. Understanding the extent and timing of in vivo events triggered by free radicals is important to consider, as these are major determinants of disease evolution and progression. By combining molecular magnetic resonance imaging (mMRI) and immuno-spin trapping (IST) technologies it is possible for the first time to monitor levels of in vivo radicals in rodent glioma models.

Numerous studies indicate that oxidative stress, a result of an imbalance in levels of ROS/RNS and anti-oxidative defense systems, plays a crucial role in cancer. Free radicals are involved and/or are the causal agents in several cancers, including gliomas. ROS/RNS may directly oxidize nucleic acids, proteins, carbohydrates and lipids, causing intracellular and intercellular perturbations in homeostasis, including DNA mutations and interference with DNA repair [1]. High concentrations of lipid-derived electrophilic products resulting from the oxidation process readily react with proteins, DNA and phospholipids, generating intra- and intermolecular toxic covalent adducts that lead to the propagation and amplification of oxidative stress [1].

Abbreviations: Anti-DMPO probe, anti-DMPO antibody–albumin–Gd-DTPA–biotin; BSA, bovine serum albumin; EDC, N-succinimidyl 3-(2-pyridyldithio)-propionate; Gd-DTPA, gadolinium-diethylene triamine penta acetic acid; HN, 4-hydroxynonenal; IgG, immunoglobulin G; IgG contrast agent, IgG-albumin–Gd-DTPA–biotin; IHC, immunohistochemistry; IST, immuno-spin-trapping; DMPO, 5,5 dimethyl-1-pyrroline-*N*-oxide; MRI, magnetic resonance imaging; mMRI, molecular MRI; MDA, malondialedhyde; NHS, N-succinimidyl-S-acetylthioacetate; 3-NT, 3-nitrotyrosine; PBS, phosphate buffer saline; ROIs, regions of interest; ROS/RNS, reactive oxygen (nitrogen) species; TE, echo time; TR, repetition time

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Free radicals generated as a result of oxidative stress processes can be trapped by 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) to form DMPO-radical adducts, which can then be further tagged by IST, a method that utilizes an antibody against DMPO-adducts [2–5]. It would be of paramount importance if the formation of oxidation products could be assessed in vivo, allowing the study of specific cause–consequence relationships from specific oxidative events. This approach would allow scientists to correlate the detection of in situ oxidative stress markers with specific longitudinal pathological conditions associated with glioma tumor growth.

In a novel approach, we have combined the desired morphological image resolution of mMRI with a Gd-DTPA–albumin-based contrast agent for signal detection with the specificity of an antibody for DMPO nitrone adducts (anti-DMPO probe), to detect in vivo free radicals (see Fig. 1). In this study, the anti-DMPO probe was used to assess heterogeneous free-radical formation within orthotopic mouse gliomas.

2. Methods

2.1. Syntheses of DMPO-specific MRI contrast agents

To recognize the DMPO-radical adducts, a mouse monoclonal anti-DMPO antibody bound to a contrast agent was used. The macromolecular contrast material, biotin–BSA–Gd-DTPA, was prepared using a modification of the method of Dafni et al. [6]. The biotin moiety in the contrast material was added to allow histological localization. Biotin–BSA–Gd-DTPA was synthesized as described in Towner et al. [7]. A solution of biotin–BSA–Gd-DTPA was added directly to the solution of antibody (anti-DMPO, 200 μg/mL) for conjugation through a sulfo-NHS (N-succinimidyl-S-acetylthioacetate)–EDC (N-succinimidyl 3-(2-pyridyldithio)-propionate) link between albumin and antibody according to the protocol of Hermanson [8]. Sulfo-NHS was added to the solution of biotin–BSA–Gd-DTPA and EDC. This activated solution was added directly to the antibody (anti-DMPO, 20 μg/mL) for conjugation. The mixture was left to react for at least 2 h at 25 °C in the dark. The product was lyophilized and subsequently stored at 4 °C and reconstituted to the desired concentration for injections in phosphate buffer saline (PBS). The final amount of the product, anti-DMPO–biotin–BSA–Gd-DTPA (anti-DMPO probe), that was injected into the mice is estimated to be 20 μg anti-DMPO Ab/injection, and 10 mg biotin–BSA–Gd-DTPA/injection. The estimated molecular weight of the anti-DMPO–biotin–BSA–Gd-DTPA probe is estimated to be 232 kDa. As a control, normal mouse-IgG (obtained from a healthy mouse population; Alpha Diagnostic International, San Antonio, TX, USA) conjugated to biotin–BSA–Gd-DTPA (control IgG contrast agent) was synthesized by the same protocol to generate an isotype contrast agent.

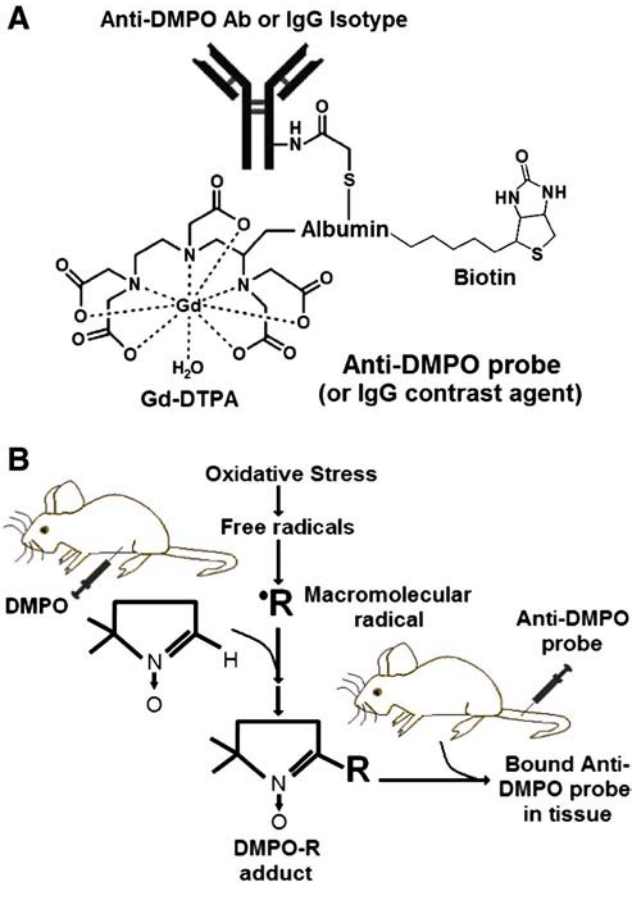

Fig. 1. Approach for combined in vivo mMRI and IST. (A) Anti-DMPO–albumin–Gd-DTPA–biotin mMRI probe (anti-DMPO probe). (B) Immuno-spin trapping of free radicals (•R) with anti-DMPO mMRI probe. DMPO is injected i.p. to trap free radicals and generate nitrone-radical (R) adducts. Anti-DMPO is injected i.v. to target nitrone-R adducts, which can be visualized by mMRI.

2.2. In vitro characterization of anti-DMPO probe

Vials were prepared containing either GL261 mouse glioma cells (10^6) alone, GL261 cells with hydrogen peroxide (H_2O_2) and DMPO, GL261 cells with DMPO + anti-DMPO probe, GL261 cells with H_2O_2 + DMPO + anti-DMPO probe, or water (no cells). Cells (mouse GL261 cells) were grown in flasks in complete growth medium (DMEM Media with 10% fetal bovine serum (FBS), Invitrogen, Grand Island, NY, USA) to confluency. Two to three hours before treatment, the growth medium was replaced with serum-free medium. DMPO (40 mM) was added to appropriate vials, and after 15 min equilibrium, H_2O_2 (50 μM) was added. In the samples that contained all components, the anti-DMPO probe was added (2 μg, based on antibody calculation), and cells were incubated for 45 min. Following incubation, cells were collected, washed with PBS, centrifuged (500 rpm), and the pellet was resuspended in PBS for MR imaging. Each measurement was repeated 4 times per treatment group.

2.3. Animal experiments

All animal experiments were conducted in accordance with the National Institutes of Health animal use and welfare guidelines, and with the authorization of the Oklahoma Medical Research Foundation institutional animal ethics committee.

2.3.1. Intracranial mouse brain tumor model

As a model for orthotopic intracranial brain tumors, a GL261 mouse glioma model was used (n = 8). Mouse glioma cells (GL261) were implanted intracerebrally in C57BL6/J mice. The heads of anesthetized mice were immobilized (stereotaxic unit; Kopf Instruments, Tujunga, CA), and with aseptic techniques, a 1 mm burr hole was drilled in the skull 1 mm anterior and 2 mm lateral to the bregma on the left side. A 20 μL gas-tight Hamilton syringe was used to inject 2×10^4 GL261 cells (in 10 μL of PBS) into the left frontal lobe at a depth of 1.5 mm relative to the dural surface in a stereotaxic unit. The cell lines were maintained and expanded immediately prior to inoculation. Following injection, the skin was closed with surgical sutures. The anti-DMPO probe was administered at 19 days following cell implantation. Mice (n = 4) were treated with DMPO for 3 days starting at day 16 following implantation of cells, and prior to the administration of the anti-DMPO probe. For an isotype contrast agent control, GL261 glioma-bearing mice (n = 4) were treated with DMPO, but were administered a non-specific mouse IgG-albumin–Gd-DTPA–biotin (IgG) contrast agent.