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***N*-[¹¹CH₃]Dimethylaminoparthenolide (DMAPT) uptake into orthotopic 9LSF glioblastoma tumors in the rat**

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Abstract

The aim of this study was to determine the uptake of intravenously administered *N*-[¹¹CH₃]dimethylaminoparthenolide (DMAPT) into orthotopic 9LSF glioblastoma brain tumors in Fisher 344 rats from positron emission tomography (PET) imaging studies. [¹¹C]Methyl iodide (¹¹CH₃I) was utilized as a [¹¹C]-labeling reagent to label the precursor methylaminoparthenolide (MAPT) intermediate. From PET imaging studies it was found that brain uptake of *N*-[¹¹CH₃]DMAPT into brain tumor tissue was rapid (30 minutes), and considerably higher than that in the normal brain tissue.

Graphical abstract

Keywords

Dimethylamino parthenolide (DMAPT); [¹¹C]-labeled DMAPT; Uptake into brain; PET-imaging

Dimethylaminoparthenolide (DMAPT, Fig. 1, **1**) is a water-soluble analog of the naturally occurring sesquiterpene lactone, parthenolide (PTL, Fig. 1, **2**).^{1–4} PTL was originally isolated from the plant feverfew (*Tanacetum parthenium*),^{5,6} and has since been shown to

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possess anticancer activity against both hematological^{2,7} and solid tumors^{1, 8–12}, but has poor water-solubility² and low oral bioavailability (~1–2%).² Other structurally related sesquiterpenes, such as melampomagnolide B¹³, and micheliolide,^{14,15} also exhibit anticancer properties, and several structural analogs of these three natural products are currently being investigated as potential anticancer agents.^{16,17} In this respect, DMAPT, a C-11 *N,N*-dimethylamino Michael addition adduct of PTL, has been shown to exhibit similar anticancer activity when compared to PTL against hematological tumor cells^{2,7} and against a wide range of solid tumors, including pancreatic,^{18–21} non-small cell lung,^{22,23} prostate,²⁴ bladder,¹ and breast cancers.²⁵ DMAPT has superior drug-like properties when compared to PTL, and is about 1000 times more water-soluble² and has good oral bioavailability (70%) in the rat.⁷

The mechanism of action of both PTL and DMAPT has been extensively investigated in recent years, and involves both inhibition of the NFκB pathway, as well as inhibition of key enzymes in glutathione biosynthesis and function, such as glutathione ligase (GCLC and GCLM) and glutathione peroxidase (GPX).^{26–28} Currently, DMAPT fumarate (LC-1) is in Phase 1 clinical trials as a first-in-class NFκB inhibitor for the treatment of acute myelogenous leukemia (AML).^{1,7}

In vitro screening of DMAPT against 9LSF-rat gliosarcoma cell lines utilizing both MTT metabolic assays and cell growth data afforded promising results on cell viability (Fig. 2). This encouraged us to study the uptake of *N*-[¹¹CH₃]DMAPT into orthotopic brain tumors by [¹¹CH₃]-labeling of the precursor molecule methylaminoparthenolide (MAPT, Fig. 1, **3**) with [¹¹C]methyl iodide (¹¹CH₃I) as radiolabeling agent and utilizing the resulting *N*-[¹¹CH₃]DMAPT in positron emission tomography (PET) brain imaging studies in Fisher 334 rats.

The molecular weight (MW = 293.40) and calculated log P value (logP = 1.55) of DMAPT suggests that this anticancer agent should be able to access the blood brain barrier after peripheral administration. Consequently, if [¹¹CH₃]DMAPT is able to enter brain tumors and be retained therein, then this would provide enough confidence to begin a study to test DMAPT efficacy in inhibiting tumor growth and/or effecting tumor regression.

The radiochemical synthesis of *N*-[¹¹CH₃]DMAPT utilized the general approach described by both Berger²⁹ and Langstrom et al.³⁰ The reaction of the precursor molecule MAPT (Scheme 1, **3**) with ¹¹CH₃I in the synthesis of *N*-[¹¹CH₃]DMAPT (**4**) resulted in good radiochemical yields of product under basic conditions, as determined by HPLC analysis.

Initially, the precursor molecule, methylaminoparthenolide (MAPT, **3**) was synthesized from PTL (**2**) via reaction with CH₃NH₂/MeOH. PTL (1.0 mmol) was dissolved in methanol (10 mL) at room temperature and methylamine in methanol (1.2 mmol) was added; the resulting mixture was stirred at ambient temperature for 4 hours (Scheme 1). Completion of the reaction was monitored by TLC. Methanol was removed by evaporation under reduced pressure on a rotary evaporator, and the desired product was precipitated from methanol as a white solid. Confirmation of structure and purity was obtained from ¹H-NMR, ¹³C-NMR and mass spectroscopic analysis. ¹H NMR (CDCl₃, 400 MHz): δ 5.20 (d, *J* = 9.6 Hz, 1H),

3.85 (t, $J = 9.2$ Hz, 1H), 2.96 (dd, $J = 3.6, 8.8$ Hz, 1H), 2.79–2.72 (m, 2H), 2.50–2.43 (m, 4H), 2.41–2.03 (m, 6H), 1.94 (dd, $J = 6.4, 14.8$ Hz, 1H), 1.72–1.64 (m, 5H), 1.29 (s, 3H), 1.25–1.17 (m, 1H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 177.4, 134.9, 124.9, 82.0, 65.9, 61.6, 49.2, 47.7, 46.1, 40.9, 37.1, 36.6, 29.4, 24.1, 17.3, 17.1 ppm, HRMS for $\text{C}_{16}\text{H}_{26}\text{NO}_3$. (MH $^+$): calcd: 280.1913, found: 280.1901.

The synthesis of N -[^{11}C]DMAPT was carried out as follows: $^{11}\text{CO}_2$ was prepared by the $^{14}\text{N}(\text{p},\alpha)$ reaction on 2% O_2 in N_2 target gas using a Siemens Eclipse 11 MeV cyclotron and the manufacturer's carbon-11 target. Carbon dioxide from the cyclotron target was condensed in 1/8 inch stainless steel tubing cooled in liquid nitrogen. After a 5 mL/min flow of helium was established through the trap to a reaction vessel, the trap was warmed to release carbon dioxide which was bubbled through a solution of 3 μmole LiAlH_4 in THF dried by distillation from sodium benzophenone ketyl to collect the carbon-11. After evaporation of the solvent, addition of 0.5 mL 57% HI solution afforded [^{11}C]methyl iodide, which was distilled through a 0.5 mL sodium carbonate trap and collected in 300 μL acetonitrile containing 1 mg MAPT and 2 micromoles sodium hydroxide. N -[^{11}C]Methylation of MAPT proceeded to afford N -[^{11}C]DMAPT in 10 min at 90 $^\circ\text{C}$. After completion of the reaction 300 μL of water were added before injection onto the HPLC unit. Solvent was evaporated from the collected product which was then taken up in saline for injection (USP) and filtered through a 0.22 micron sterile filter into a sterile vial for administration in animal studies. N -[^{11}C]DMAPT was obtained in 20% yield from carbon dioxide, 25% yield from methyl iodide and in 3% overall radiochemical yield at the end of bombardment [(EOB) 60 min]. Product specific activity was determined to be 1–2 Ci/ μmole .

In the Micro-PET imaging studies with N -[^{11}C]DMAPT in rats, orthotopic gliosarcoma tumors were grown in Fisher 344 rats using the San Francisco variant of the rat 9L gliosarcoma cell line.^{31,32} Rats were anesthetized, placed into a stereotactic holder and a hole was drilled through the skull, above the region of the brain selected for placement of the tumor (using a rat brain stereotactic atlas). A syringe with a 25 g needle, was attached to the stereotactic manipulator and was inserted into the brain to deliver 1×10^5 tumor cells (suspended in 5 μL of Matrigel) to initiate a tumor either in the cerebral cortex or within subcortical white matter. MRI (7 T Bruker Instrument) was used to monitor tumor growth, and rat brain images taken 2–12 h prior to the MicroPET images served as a high resolution morphological reference for the subsequent MicroPET images.

Rats were placed onto the Siemens Focus 220 Micro-PET and 1 mCi of N -[^{11}C]DMAPT was injected either intravenously (IV) into the tail vein or mixed with a 40 mg/kg dose of cold DMAPT and injected intraperitoneally (IP) into Fisher 334 rats. Micro-PET imaging was initiated immediately following injection of the N -[^{11}C]DMAPT. Pharmacokinetic data for N -[^{11}C]DMAPT within tumor and normal brain tissue are reported as Standardized Uptake Values (SUV).

Figure 3 presents representative experimental results obtained when a 9L-SF tumor bearing rat was injected with 1.0 mCi of N -[^{11}C]DMAPT (0.61 μmoles) in the tail vein. The N -[^{11}C]DMAPT was brought to 100 μL with sterile, normal saline, injected and 100 μL of

saline was administered intravenously (IV) afterwards to ensure that all of the *N*-[¹¹CH₃]DMAPT was introduced into the bloodstream. Both the Micro-PET (Fig. 3A) and MRI (Fig. 3B) images show the maximal cross sectional area of the tumor and the graph in Fig. 3C illustrates the temporal variation of the SUV, recorded within regions of interest (ROI), located within the tumor (tumor volume 118.94 mm³) and within the normal brain tissue.

When 1.0 mCi (0.74 μmoles) of *N*-[¹¹CH₃]DMAPT was mixed with a 40 mg/kg dose of cold DMAPT and injected into the rat intraperitoneally (IP), as with IV delivery, more *N*-[¹¹CH₃]DMAPT entered and was retained within the tumor than in the normal brain tissue (Fig. 4). However, Fig. 4C shows that the buildup of the *N*-[¹¹CH₃]DMAPT within the tumor occurred more slowly and was then retained substantially longer than following IV delivery.

As in the IV administered *N*-[¹¹CH₃]DMAPT experiments, the perimeter of the tumor received a substantially lower dose of *N*-[¹¹CH₃]DMAPT compared to the central tumor regions when the drug was administered IP. It is also clear from Fig. 4B that high levels of *N*-[¹¹CH₃]DMAPT accumulated within the eyes and nasal pharynges of the rat.

The Micro-PET data demonstrate clearly the most important result of this study; viz. DMAPT preferentially enters into and is retained within the brain tumors. Other important factors need to be worked out more precisely to measure the pharmacokinetics of DMAPT in this rat brain tumor model with greater quantitative precision and accuracy. The most difficult of these is correcting for the partial volume effect,³³ which is especially difficult to manage for Micro-PET imaging of such small tumors. That is because the size of the imaged tumors is similar to the resolution limit of the MicroPET (1.5–3.0 mm). Hence, the ROIs used to calculate the SUV values will contain radioactivity level data from surrounding normal tissue, which will reduce the apparent SUV for the tumor. This effect will be greater when larger ROIs are used to measure the tumor SUV, as illustrated in Fig. 4C. It should be noted that, because of the partial volume effect, the amount of *N*-[¹¹CH₃]DMAPT (and therefore DMAPT) within the tumor is almost certainly greater than the highest values measured in the ROIs. Hence, the measured values shown in Figs. 3 and 4 arguably represent lower limits for *N*-[¹¹CH₃]DMAPT penetration into the tumors. Regardless, the MicroPET data presented herein provide sufficient support for moving forward with DMAPT treatment efficacy studies, during which experimental issues, such as the partial volume effect, can be worked out more rigorously.

The preferential, tumor uptake of DMAPT appears to occur in a manner that is consistent with other drugs and therapeutics that exhibit the enhanced permeability and retention (EPR) effect for tumors.^{34,35} This is an important property of the DMAPT molecule regarding it being considered as a potential treatment for human brain tumors. It will be important to determine if the EPR activity of DMAPT is limited to the characteristic leakiness of the tumor vasculature within the more central regions of the tumor, or if DMAPT is somehow capable of penetrating the blood brain barrier and being retained preferentially within tumor cells, even in the tumor perimeter where the vasculature has more normal permeability characteristics.

The paired Micro-PET and MRI images in Fig. 3 were selected such that each presented as close to the same region of the brain as possible. Thus, based upon these representative images, and what has been reported in the literature, the EPR effect for DMAPT was likely most effective in the central tumor regions where, in general, more permeable blood vessels are to be found. While an EPR effect mediated by leaky tumor vasculature represents a pathway for preferential DMAPT delivery into brain tumors, it may not lead to uniform distribution of the drug throughout the tumor. Either greater DMAPT dosage regimens, longer delivery times, multiple fractionated doses or a combination of these three factors may be required to deliver therapeutically effective levels of DMAPT throughout these brain tumors.

The coronal image in Fig. 4B illustrates that the nasal pharynxes and the eyes are body tissues that absorb/adsorb DMAPT more readily than the brain tumors. The salivary glands also accumulated higher levels of N -[$^{11}\text{CH}_3$]DMAPT (see graphical abstract). The 400-fold more massive amount of cold DMAPT (26.4 mmoles) delivered with the N -[$^{11}\text{CH}_3$]DMAPT, and the slower release into the bloodstream following IP injection may have saturated blood and tissue absorption/adsorption sites of DMAPT early on and eventually permitted more of later-delivered N -[$^{11}\text{CH}_3$]DMAPT to remain unbound and capable of accumulating in the brain tumor, which may contribute to the more sustained level of N -[$^{11}\text{CH}_3$]DMAPT within the tumor that is illustrated in Fig. 4C.

The preferential accumulation of DMAPT in the salivary glands, nasal pharynxes and the eyes may offer some other pharmaceutical applications for this interesting anticancer agent. This would be especially true for any cancers that occur in these tissues; but only if DMAPT's remarkable selective toxicity for tumor cells over normal cells, which has been reported for acute myeloid leukemia (AML)²⁸ and other cancers,³⁶ holds true for these tumors.

Regardless of where a solid tumor is located, using a strategy of repeated, fractionated doses of DMAPT may result in more of the drug being delivered into and retained within the tumor center and eventually diffusing outward to deliver a therapeutically significant dose to the tumor periphery. Other approaches, including targeted nanoparticles, might be required to deliver effective DMAPT doses throughout the entire tumor to produce the desired therapeutic effect of inhibiting tumor growth.

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Fig. 1.
Dimethylaminoparthenolide (DAMPT; **1**), parthenolide (PTL; **2**) and
methylaminoparthenolide (MAPT; **3**).

Fig. 2.

Growth inhibition of 9LSF rat gliosarcoma cells by dimethylaminoparthenolide (DMAPT). Cells were seeded into 35 mm culture dishes (cell counts) or 96 well plates (MTT assay) so as to reach confluence in 72 h without any drug treatment (NCI protocol). Drug was then added 24 h later at the rate of 0.333 mL of drug-containing medium per cm² of growth area. Cell counts and the MTT assay were performed 48h after drug treatment was initiated.

Fig. 3. N -[$^{11}\text{CH}_3$]DMAPT MicroPET (A), MRI (B) and pharmacokinetic data (C) following tail vein injection of N -[$^{11}\text{CH}_3$]DMAPT.

Fig. 4. N -[^{11}C]DMAPT Micro-PET Axial (A) and coronal (B) images; and pharmacokinetic data (C) following intraperitoneal injection of N -[^{11}C]DMAPT mixed with 40 mg/kg of unlabeled DMAPT. Using a larger ROI to calculate the SUV within the tumor (TumorLarge) resulted in a significant reduction in the differential between tumor and normal tissue SUV, a result of the partial volume effect.

Scheme 1.

Synthesis of N -[$^{11}\text{CH}_3$]- N,N -dimethylaminoparthenolide (N -[$^{11}\text{CH}_3$]DMAPT) from parthenolide (PTL)