Annals of Case Reports
Zeira O, et al. Ann Case Rep: 9: 102138
www.doi.org/10.29011/2574-7754.102138
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Case Report

Cases Report on Innovative Drug-Delivery System Containing Paclitaxel for Treating Canine Gliomas

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Citation: Zeira O, Ghezzi E, Pettinari L, Devoti C, Pia Dumas M, et al (2024) Cases Report on Innovative Drug-Delivery System Containing Paclitaxel for Treating Canine Gliomas. Ann Case Report. 9: 2138. DOI:10.29011/2574-7754.102138

Received: 18 December 2024, **Accepted:** 23 December 2024, **Published:** 26 December 2024

Abstract

In human and veterinary medicine, tumors such as glioblastoma represent an unfavourable diagnosis with short median survival. This study aims to evaluate a biological drug delivery system, micro fragmented adipose tissue (MFAT), that allows a slow release of paclitaxel (PTX) into spontaneously occurring gliomas in owned dogs. Methods: The study involved intratumoral treatment of gliomas in dogs using MFAT combined with PTX. The pharmacokinetic profile of the treatment was assessed, and quality-of-life scores were recorded. MRI scans and 3D rendering were used to measure tumor volume changes. Histological analysis was performed to evaluate the effects of the treatment on tumoral mass and surrounding brain tissue. Results: The intratumoral treatment showed no short- or mid-term adverse effects. The quality-of-life score was good in most cases. MRI scans and 3D rendering assessment revealed a decrease in tumor volume in 5 out of 6 dogs treated with MFAT+PTX, whereas all 3 dogs in the control group treated with temozolomide or lomustine showed continuous tumor volume increase. Histological analysis demonstrated that MFAT+PTX caused necrosis of the tumoral mass and a reactive glial-mesenchymal response, with no signs of neurotoxicity in the brain tissue apart from the treated focal tumor site. Conclusions: These preliminary results suggest that MFAT+PTX treatment may be a promising approach for glioma treatment in dogs, warranting further investigation with a larger group of canine patients. Moreover, these findings provide a proof of concept, demonstrating the safety and feasibility of this approach for potential human translational applications.

Keywords: Canine gliomas; Glioblastoma; Drug delivery system; Paclitaxel; Micro fragmented adipose tissue

Introduction

Glioblastomas in humans remain highly lethal, with a median survival of 12-14 months despite available treatments like surgery, radiotherapy, and chemotherapy, resulting in a mortality rate exceeding 90% within one-year post-diagnosis. Although significant preclinical research has aimed at enhancing the quality of life and overall survival, the standard treatment approach continues to rely on the Stupp Protocol [1]. Despite advancements in surgical techniques, neuronavigation, intraoperative monitoring, radiosurgery, chemotherapy, immunotherapy, and other methods, no substantial improvements in survival outcomes have been achieved over the past four decades, with many promising preclinical therapeutic strategies failing to progress beyond phase I/II clinical trials.

Among different possible therapeutic approaches, the development of drug delivery systems (DDS) to enhance drug accumulation within tumors has been suggested. DDS have demonstrated potential in mitigating the primary challenges of chemotherapy, such as poor bioavailability, high dosage requirements, adverse side effects, low therapeutic indices, and the development of drug resistance [2]. Innovations in materials science, including polymer drug conjugates, liposomal systems, transdermal patches, controlled-release microchips, and nanotechnology, have further propelled research in this area, offering the potential for improved chemotherapeutic regimens. However, the clinical applicability and safety of these techniques remain under scrutiny.

Among the various DDS explored, micro fragmented adipose tissue (MFAT) has emerged as a promising biological medium for drug delivery. MFAT enables the slow release of chemotherapeutic agents adjacent to tumors, potentially enhancing therapeutic effects while reducing systemic side effects [3-5]. Composed of stromal vascular fractions, pericytes, and adipose-derived stem cells, MFAT exhibits trophic, mitogenic, anti-scarring, anti-apoptotic, immunomodulatory, and antimicrobial properties, making it an attractive option for drug delivery [6-8]. Our previous studies demonstrated that MFAT can be effectively loaded with paclitaxel (PTX), a plant-derived alkaloid used in various solid tumors [3,4], despite the challenges posed by hypersensitivity and other side effects [13].

In brain tumors, the blood-brain barrier (BBB) poses a significant obstacle to the delivery of systemic drugs, including PTX [14]. Local DDS, such as biodegradable wafers loaded with carmustine, have been investigated to circumvent the BBB, but their impact on survival in high-grade gliomas has been limited. They are often accompanied by severe side effects, including infections and increased intracranial pressure [15]. Alternative approaches, like low-intensity pulsed ultrasound combined with microbubble injection, have been proposed to transiently disrupt the BBB, enhancing drug delivery to the brain, though clinical efficacy remains unproven [16-19].

Spontaneous canine brain tumors have gained recognition as valuable translational models for human brain tumor research. Building on our previous MFAT-PTX studies [3-5], this study investigates the safety and efficacy of intratumoral PTX-loaded MFAT in dogs with gliomas. Canine gliomas, representing over one-third of primary brain tumors, are aggressive and poorly responsive to current treatments, leading to a median survival of only 2-3 months [20-29]. This study is the first clinical application of a PTX-based biological DDS in dogs with intra-axial brain tumors, demonstrating both safety and efficacy. The findings suggest that MFAT-PTX may be a promising therapeutic approach in veterinary oncology, with potential translational applications in human medicine.

Materials and Methods

Dog Population and Selection Criteria

Nine dogs with spontaneously developed brain gliomas were included in this study, conducted at the San Michele Veterinary Hospital, Tavazzano con Villavesco, Italy, between 2019 and 2021. The cohort comprised 3 Boxers, 3 French Bulldogs, 1 Labrador Retriever, 1 Crossbreed, and 1 Golden Retriever, with six males and three females, aged between 6 and 10 years and weighing from 8 to 37 kg. Inclusion criteria were based on pre-mortem clinical diagnosis, including history, neurological signs (e.g., seizures, behavioural changes, mentation, gait abnormalities, proprioception deficits, head tilt, circling, head pressing and visual impairment) and MRI-confirmed tumor location [22,30,31]. Cytopathological confirmation via fine needle aspiration was mandatory before treatment, followed by histopathological analysis on biopsy specimens taken prior to the intra-tumoral treatment and repeated post-mortem. Additional criteria included an age limit of 12 years, total body (TB) CT scans to exclude other comorbidities and signed written consent from the owners.

Six dogs (cases 1-6) were randomly assigned to the treatment group, while three dogs (cases 7-9) served as controls (CTRL), receiving standard therapies. CTRL dogs were treated with either temozolomide (100 mg/m² for 5 days every 3 weeks for four cycles) or lomustine (60 mg/m² every 3 weeks for four cycles) and all were administered prednisone (0.5 mg/kg/day) for the first two months post-diagnosis (Table 1).

Case	Breed	Sex	Age (years)	Body weight (kg)	Main neurological sign	Tumor localization	Histology	Therapy
1	Boxer	M	8	37	Seizures	Left olfactory- frontal lobe	ODG grade II	MFAT+PTX
2	Crossbreed	M	8	8	Seizures	Right olfactory- frontal lobe	ODG grade II	MFAT+PTX
3	Boxer	Fs	10	27	Seizures	Right temporo- occipital lobe	ODG grade II	MFAT+PTX
4	French Bulldog	M	6.5	15	Seizures	Left piriform lobe	ODG grade II	MFAT+PTX
5	French Bulldog	M	8	17	Seizures	Right Brainstem	ODG grade II	MFAT+PTX
6	Labrador Retriever	M	10	35	Seizures	Right and left olfactory- frontal lobe	ODG grade II	MFAT+PTX
7	French Bulldog	Mn	9	16	Seizures	Right olfactory- frontal lobe	ODG grade II	Lomustine
8	Boxer	Fs	6	26	Seizures	Left frontal lobe	ODG grade II	Lomustine
9	Golden Retriever	F	7	30	Seizures	Left fronto- parietal lobe	ODG grade II	TMZ

Legend: F= female, Fs=female spayed, M=male, MFAT+PTX=Microfragmented Adipose Tissue + Paclitaxel, Mn=male neutered, ODG=oligodendroglioma, TMZ=Temozolomide. Cases 1-6: treated by MFAT+PTX with drug dosages ranging from 5 to 17 ug/kg based of the body weight. Cases 7-9: control group, treated by conventional chemotherapy + prednisone 0.5mg/kg/day in the first 2 months after diagnosis.

Table 1: Basic patients' information.

Preoperative and Postoperative Clinical and Radiological Work-up

Before surgery, all dogs underwent a complete blood count (CBC), serum biochemistry, and cerebrospinal fluid (CSF) collection from the cisterna magna. CSF analysis involved cell counting using a Burker chamber, cytological evaluation of air-fixed May-Grünwald Giemsa-stained sediment and protein concentration measurement.

Preoperative MRI scans were conducted using a 0.25-Tesla magnet (Vet MR Grande, Esaote). The MRI protocol included nine sequences: transverse and sagittal T2-weighted FSE, dorsal fluid attenuated inversion recovery (FLAIR), dorsal Hyce 3D, T2*-weighted GE, T1-weighted SE transverse and T1-weighted dorsal 3DGE. Gadolinium-based contrast enhancement (0.3 ml/kg) was administered intravenously and the last two sequences were repeated post-contrast.

In order to assess tumor response to treatment, a quantitative evaluation using semi-automatic segmentation of post-contrast T1-weighted dorsal 3DGE 0.3mm slice images was performed with ITK-SNAP software [32] and 3D rendering was used for visualization. Tumor volume was assessed at 4-6 weeks postoperatively, followed by evaluations every 8-10 weeks. Early postoperative MRI was excluded to avoid interpretation difficulties due to potential artifacts from the injected material. Concurrent total body CT scans were conducted to exclude disseminated lesions. Ultrasound (US) imaging was periodically used for brain assessments, facilitated by a synthetic prosthesis covering the surgical bone defect, which was transparent to US waves.

Tumor response was categorized using a simplified version of the RANO criteria for gliomas [33] with classifications as follows: complete remission (CR): 100% decrease; partial decrease (PR): ≥50% decrease; minor response (MR): 25%-50% decrease; stable disease (SD): <25% change; progressive disease (PD): >25% increase.

Postoperative follow-up included neurological examinations and blood tests every two weeks until the dog's death.

Treatment Protocol

The preparation of micro fragmented adipose tissue (MFAT) followed a standardized protocol [3,34]. Adipose tissue was harvested via lipoaspiration from the lumbar flanks of a donor dog. The tissue was then micro fragmented using a dedicated device [3,34-36], which allowed minimal manipulation without

enzymatic procedures. The MFAT samples were cryopreserved at -80°C and later used as a scaffold for loading paclitaxel (PTX). Prior to the biopsy, MFAT was thawed and loaded with PTX (1 mg/ml) by stirring for 30 minutes as previously described [3]. Dogs were premedicated and anesthetized as described earlier. A neuronavigation-planned craniotomy (Stealth Station S7 – Medtronic) was performed to create a skull window closest to the tumor location. Following dural opening, an ultrasound-guided fine needle aspiration biopsy was performed for histological evaluation. Subsequently, MFAT-PTX was injected slowly and controlled into the center of the tumor at the same location where the biopsy tissue was collected. Injection volumes ranged from 0.4-0.5 ml, depending on tumor size, as determined by MRI-based 3D rendering. Post-injection, the craniotomy site was covered with a synthetic, biocompatible prosthesis, designed to facilitate future monitoring and repeat injections (Figure 1). Prosthesis positioning was confirmed via postoperative CT scan.

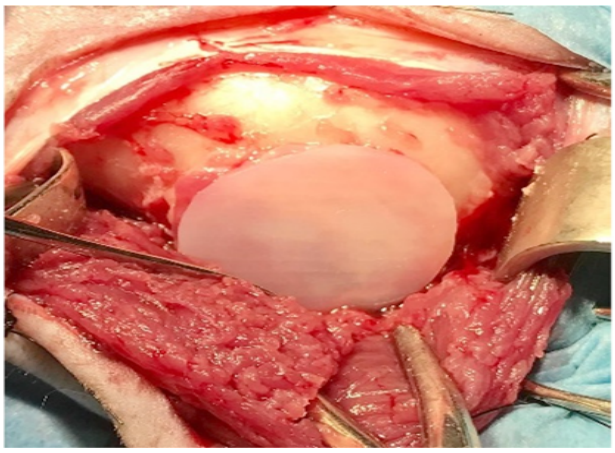

Figure 1: Craniectomy site covered by the synthetic, biocompatible prosthesis, useful for periodic brain controls being completely transparent to US waves.

Pharmacokinetics (PK) Study

Pharmacokinetic parameters (Cmax, Tmax, T1/2, Vd and AUC) were determined by measuring PTX levels in the blood at baseline (T0) and at intervals of 0.5, 1, 3, 24, 72 and 168 hours post-treatment. Residual PTX levels in the tumor were also measured post-mortem. PTX concentrations and its hydroxylated metabolites were quantified using a 3200 qTRAP system, with LC and MS optimized for separation, detection, identification and quantification.

Follow-up and Side Effects

All dogs were followed until death, either due to the tumor or unrelated causes, as owners consented to continued diagnosis and treatment. Periodic clinical and radiological assessments were performed to monitor tumor behavior and overall health. All observed side effects, both minor and major, were documented in detail. Owners provided weekly reports on their dogs' status, covering appetite, excretion, pain, neurological abnormalities and behavior. They also rated the quality of life on a scale from 1 (poor) to 4 (excellent). Survival time was defined from the date of MRI diagnosis to death. Post-mortem, all dogs underwent histological and immunohistochemistry (IHC) examinations of the brain.

Histology and Immunohistochemistry (IHC)

Brains were removed post-mortem, fixed in 4% buffered formalin and processed for paraffin embedding. Four-micron thick histological sections were prepared and stained with hematoxylin-eosin (HE) and Luxol Fast Blue (LFB). The most representative tumor sections were also stained using the immunoperoxidase method with markers including Olig2, GFAP, Vimentin, VWF, and Ki-67 (Supplementary Table 1). Whole slide imaging was obtained using a digital slide scanner (NanoZoomer S60, Hamamatsu Photonics Italia S.r.l.), with image adjustments for brightness, contrast and color balance performed using NDP.view2 software (Hamamatsu Photonics K.K.). Tumor classification followed the criteria established in the 2018 "Revised Diagnostic Classification of Canine Glioma" [21].

Results

Dog Population and Follow-up

This study included nine dogs diagnosed with spontaneous brain gliomas, consisting of six males and three females, with a median age of 8.2 years and an average weight of 21.7 kg. Brachycephalic breeds were predominant, making up 66% of the cohort, which is consistent with the expected prevalence in this population [20,22]. The most common tumor locations were the fronto-olfactory, temporal and parietal lobes of the brain. Neurological signs primarily included isolated seizures and abnormal mentation, though other symptoms such as behavioral abnormalities, proprioception deficits, head tilt, circling, head discomfort and visual impairment were also observed.

All patients were followed until the end of their lives, ensuring no loss to follow-up. Five of the dogs (cases 1-5) underwent pre- and post-treatment MRIs, with follow-up durations ranging from a minimum of 45 days (case 4) to a maximum of 510 days (case 1). One dog (case 6) could not be fully evaluated due to death from acute aspiration pneumonia 10 days post-treatment, though its data contributed to the pharmacokinetic (PK) study. In the control group (CTRL), follow-up durations ranged from 154 days (case 9) to 207 days (case 8), with clinical evaluations, blood work-ups and postmortem neuropathology assessments completed for all.

Preoperative Radiological Data

Preoperative MRI scans revealed that most tumors were heterogeneous, characterized by T1 hypointensity and T2 and FLAIR hyperintensity, with clear mass effects and ventricular distortion. Six dogs (cases 1, 2, 5-7, 9) had deep-seated lesions. Mild to moderate contrast uptake was seen in all but one dog (case 9), while five dogs exhibited partial or complete ring enhancement (cases 1-4, 8). Mild to moderate edema was present in five dogs (cases 1, 3, 6, 7, 9). Notably, all dogs exhibited varying degrees of cystic areas within their tumors.

Post-Treatment Radiological and Clinical Assessment

Post-treatment MRI scans were used to evaluate tumor behaviour (Figure 2), with assessments conducted from the initial time point (time 0) through the last follow-up. 3D rendering analysis was employed for six dogs treated with MFAT-PTX and one dog treated with lomustine (case 7). After 40 days from the initial MFAT-PTX treatment, five of the six treated dogs showed tumor volume reduction: three dogs (cases 1-3) had reductions exceeding 50%, one dog (case 5) had a 45% reduction and one dog (case 4) had a 25% reduction. Unfortunately, case 6 passed away 10 days after treatment due to pneumonia and did not have a follow-up MRI.

Further MRI evaluations at 80 days showed additional tumor volume reductions in two cases: 32% in case 1 and 60% in case 2. Case 3, however, experienced a 92% increase in tumor volume compared to the previous MRI, necessitating a repeat MFAT-PTX treatment. Of the three remaining dogs in the treatment group, one was euthanized due to uncontrolled seizures and two died from unrelated causes. MRI scans at 120 days revealed further tumor volume decreases in cases 1 and 3, with case 1 showing a 56% reduction at 160 days, while case 3 showed a significant increase in tumor volume (+182%). Despite the tumor growth, case 3's general and neurological status remained stable until euthanasia at 245 days due to recurrent seizures. Case 1 demonstrated a complete tumor remission at 200 days, although a mild recurrence was observed at 365 days, leading to a second MFAT-PTX treatment (Figure 3). This case remained clinically stable until euthanasia due to severe paraparesis related to lumbosacral stenosis, with no significant tumor regrowth observed until then.

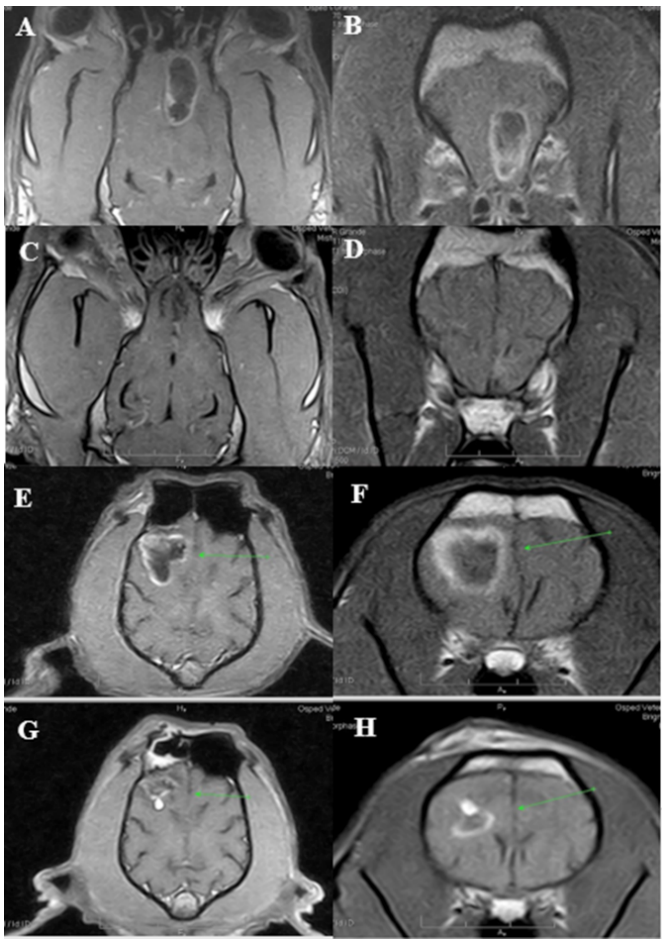

Figure 2: (A-D) Case no. 1 Pre-treatment T1 axial and coronal contrast enhanced MRI (A-B) and 6 months post-treatment (C-D); (E-H) Case no. 2 Pre-treatment T1 axial and coronal contrast enhanced MRI (E-F) and 40 days post-treatment (G-H).

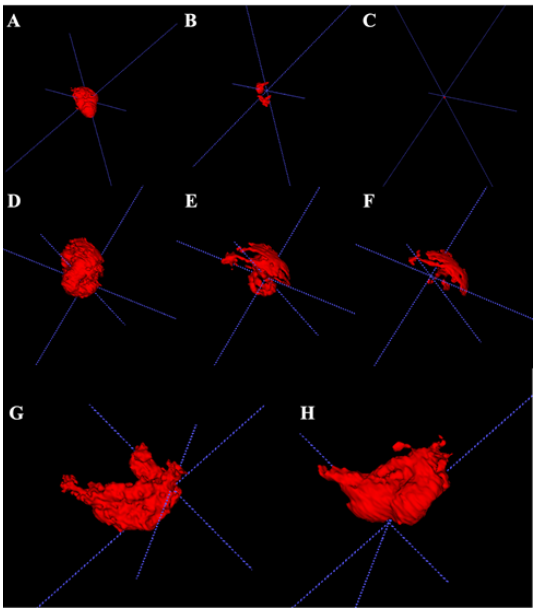

Figure 3: (A-C) Case no. 1. Sequence of tumor volumetric behavior from T0 (2190mm³) to T5 (1mm³), 200 days, single treatment; (D-F) Case no. 2. Sequence of tumor volumetric behavior from T0 (2047 mm³) to T2 (369. mm³), 70 days, single treatment; (G-H) Case no. 7. Tumor volumetric behavior T0 – T1 (120 days). Treated by conventional first-line chemotherapic drug (lomustine) and corticosteroid therapy.

In the control group, MRI scans consistently showed tumor volume increases. For instance, case 7, treated with lomustine, exhibited progressive disease, as demonstrated by 3D rendering assessment (Fig.2 G-H). Table 2 summarize tumor volume assessment and disease progression.

. Case	T0 tumor volume (Pre-treat.)	T1 40 days	T2 80 days	T3 120 days	T4 160 days	T5 200 days	T6 240 days	T7 270 days	T8 280 days	T9 375 days	Survival time (days)	Cause of Death
1	2190.95 mm³	269.75 mm³ -88% PR	183.6 mm³ -92% MR	156.89 mm³ -93% SD	68.31 mm³ -97% PR	1.49 mm³ -100% CR	128.8 mm³ -94% SD	148.9 mm³* -93% SD	632.5 mm³ -71% MR	624.7 mm³ -70% SD	510	Euthanasia due to para-paresis
2	2049.27 mm³	930.73 mm³	369.01 mm³	Deceased							90	Anesthesia complications
3	1361.74 mm³	583.37 mm³	1119.97 mm³ **	993.85 mm³	2806 mm³	Deceased					245	Euthanasia due to uncon-troled seizures
4	1445.85 mm³	1082.19 mm³ ***	Deceased								45	Euthanasia due to uncon-troled seizures
5	867.1 mm³	474.3 mm³	Deceased								50	Spontan. death, unknown reason

6	2323.1 mm³	Deceased				10	Aspiration Pneumonia
7 [c]	2707.64 mm³	NP	NP	3967 mm³ +47% PD	Deceased	139	Euthanasia due to uncontroled seizures
8 [c]	3D render. assess. NP	NP	NP	NP	Deceased	207	Euthanasia due to neurolog. signs of relapse
9 [c]	3D render. assess. NP	NP	NP	NP	Deceased	154	Euthanasia due to neurolog. Signs of Relapse
Cases 1-6 MFAT-PTX therapy, *Second treatment dog 1, **second treatment dog 3, ***second treatment dog 4. Cases 7-9 [c] control group (conventional chemotherapy). Legend: CR=complete response, PR=partial response, MR=minor response, SD=stable disease, PD progressive disease. NP=not performed.							

Table 2: Tumor volume assessment and disease progression.

Post-Treatment Clinical Assessment

Post-treatment clinical evaluations were based on weekly neurological examinations and owner-reported assessments, focusing on clinical signs, physiological functions and quality of life.

Abnormal neurological symptoms resolved within three weeks post-MFAT-PTX injection in three dogs (cases 1-3). Case 4, which had one seizure per week before treatment, showed no significant change post-treatment. Case 5 exhibited persistent depressed mentation and passed away 50 days post-treatment, likely due to brainstem tumor localization. Case 6 was evaluated only during the first 10 days post-treatment before succumbing due to pneumonia. Moreover, Owners reported normal appetite, excretion, behaviour and no visible pain in cases 1 to 5. Neurological abnormalities were absent in cases 1, 2, and 3. Four dogs were reported to have an excellent quality of life (score 4). The owner of case 4 rated the dog's quality of life as poor (score 1) due to ongoing seizures, despite other normal physiological and neurological functions. The owner of case 5 also reported lethargy and rated the dog's quality of life as acceptable (score 2). Overall, three out of six dogs treated with MFAT-PTX had a very good clinical response, maintaining a normal life with well-controlled seizures.

Safety Analysis

The MFAT-PTX treatment group showed no significant short- or mid-term adverse effects related to either the injection procedure or the drug delivery system. The injections were well-tolerated without reports of acute toxicity or hypersensitivity. Case 6's death 10 days post-treatment was attributed to unrelated causes (acute aspiration pneumonia). CBC and biochemistry profiles conducted biweekly revealed no abnormalities, indicating no systemic myelotoxicity, hepatic or renal toxicity.

In contrast, two dogs treated with lomustine in the control group experienced side effects, including neutropenia, anemia, thrombocytopenia (case 8) and vomiting (cases 7 and 8). No adverse effects were observed in the dog treated with temozolomide. Pre- and post-treatment CSF evaluations revealed no significant abnormalities except for a mild protein increase in cases 1 and 3, 40 days post-injection. No complications were associated with the surgical procedure.

Survival Analysis

The median overall survival for the first five dogs treated with MFAT-PTX was 188 days. Cases 1, 2, and 3 survived for 510, 90, and 245 days, respectively. It is noteworthy that case 2 died during anesthesia for an MRI control at 90 days post-treatment, despite being in good general and neurological health. Case 4 was euthanized 45 days post-treatment due to the owner's concerns about the dog's quality of life (seizures). Case 5 died 50 days post-treatment while sleeping. Case 6 died 10 days post-treatment from aspiration pneumonia. In contrast, the three control group dogs experienced tumor volume increases and did not show significant clinical remission, with a median overall survival of 167 days.

Pharmacokinetic (PK) Study

Pharmacokinetic analysis revealed variations in Cmax and Tmax parameters among the treated dogs, but a consistently high volume of distribution (Vd) indicated low systemic drug presence and a preference for retention in extravascular compartments. This lipophilic nature of PTX, combined with its regional administration via MFAT-PTX, likely facilitated increased drug distribution in areas with higher lipid density. These findings, coupled with

low systemic exposure, suggested minimal systemic toxicity, which was clinically observed. Notably, significant residual PTX amounts were found in post-mortem tumor samples: 9.23 pg/mg two months post-treatment in case 3 and 32.4 pg/mg four months post-treatment in case 1. Additionally, case 1 had a high residual PTX concentration (744 pg/ml) in the CSF.

Histology and IHC

Histological examinations showed that tumors were space-occupying lesions with a translucent, gelatinous appearance, pinkish-grey in colour and soft consistency, often associated with cavitation, necrosis and hemorrhage. Adjacent neuroparenchyma was moderately compressed, with perilesional edema and midline structure shifts. Tumors exhibited dense neoplastic cell proliferation, moderate pleomorphism and low mitotic activity, with limited focal infiltration (Supplementary Table 2). The vascular network was composed of thin-walled capillaries separating clusters of neoplastic cells. Nuclear immunoreactivity for the Olig2 marker was observed across all tumors, indicating grade II oligodendroglioma.

Lipid vacuoles consistent with MFAT remnants were observed within the neoplasms, surrounded by necrotic areas and hemorrhagic foci. Gliosis, reactive astrocytes, and mineralization were present at the periphery of these areas along with macrophages containing hemosiderin and hematin. Granulomatous foci with cholesterol crystal deposits were noted in cases 1 and 2, while case 2 also exhibited macrophage aggregates and gliomesenchymal proliferation near MFAT remnants. In case 6, isolated Olig2-positive neoplastic cells were detected with MFAT remnants infiltrated by neutrophils and surrounded by necrotic tissue and fibrin depositure (Figure 4).

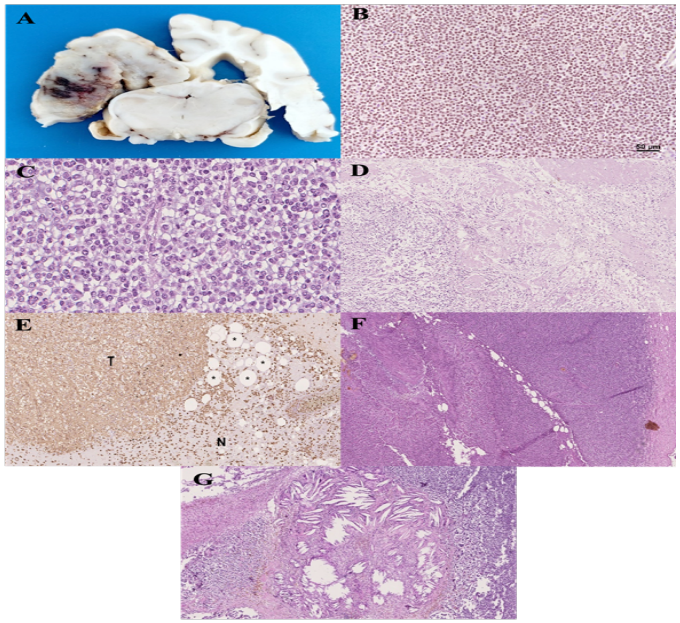

Figure 4: (A-B) Case no. 3. Extensive and compact tumor in the right cerebral hemisphere. Nuclear positivity of the neoplastic cells with the Olig2 marker (IPS Olig2, 20x); (C-D) Case no. 2. Neoplastic proliferation of cells with homogeneous nuclei, surrounded by clear cytoplasm demarcated by a distinct cell membrane (HE, 360x). Gliomesenchymal reactivity and presence of numerous macrophages with foamy cytoplasm and MFAT residues (HE, 85x); (E-F) Case no. 5. Neoplastic cell proliferation (T) is accompanied by reactive astrocytes and is surrounded by a necrotic area (N) with MFAT residues (asterisks) and numerous macrophages (IPS Vimentin, 70x). MFAT residues ("bubbles") left along the needle path after intra-tumoral MFAT-PTX injection (HE 40x); (G) Case no. 1. Granulomatous reactivity with deposit of needle-like material (cholesterol), associated with macrophages laden with foamy material and granules of hemosiderin (HE, 40x).

Discussion

Gliomas represent a heterogeneous group of brain lesions, with higher-grade variants being particularly aggressive and associated with poor prognosis due to the lack of effective treatments. In veterinary medicine, even low-grade gliomas, which are less aggressive than their high-grade counterparts, ultimately result in fatal outcomes for all patients. This study presents preliminary evidence on the safety and effectiveness of using micro fragmented adipose tissue (MFAT) loaded with paclitaxel (PTX) as a local treatment in a canine brain glioma population, suggesting the potential for this technology's application in human patients.

Effective pharmacological treatment of brain tumors requires sufficient permeability of the blood-brain barrier (BBB) and adequate drug concentrations within the tumor. However, the BBB often limits drug delivery and achieving therapeutic concentrations can lead to significant toxicological side effects. Local therapies offer a promising alternative by potentially bypassing the BBB and reducing the systemic toxicity associated with traditional drug administration. Local drug delivery methods, though promising, present challenges such as variable drug distribution and concentration, with neurotoxicity being a major dose-limiting factor [13]. Several studies have explored intra-tumoral drug delivery in both rodent and canine glioma models. Approaches such as oncolytic viral therapy, convection-enhanced delivery of liposomal CPT-11, EGFRvIII-antibody bio-conjugated magnetic iron oxide nanoparticles, and temozolomide-loaded microcylinders have shown varying degrees of efficacy with minimal adverse effects [26,37-39]. However, earlier attempts at local DDS, such as carmustine wafers, have been associated with significant side effects, including brain edema, seizures, infection, and intracranial hypertension [40,41]. In contrast, biological mediums like MFAT, due to their mechanical and chemical similarities to brain tissue, may offer a safer and more effective option for interfacing with the tumor.

Recently, MFAT has been proposed as a potential biological DDS that can provide a slow release of chemotherapeutic drugs directly adjacent to the tumor tissue [3,5]. MFAT has shown the ability to uptake and release various chemotherapy drugs, with most research focusing on PTX [3]. Despite the known hypersensitivity and toxicity associated with PTX and its co-solvents [13], the drug remains effective in treating various tumors through both systemic and subcutaneous injection [9-12]. However, PTX's effectiveness in treating gliomas is limited by poor penetration across the BBB [14]. It is known that PTX as a single chemotherapeutic regimen for canine gliomas has been tried in the past; however, its toxicity in dogs resulted to be unacceptable [42]. Conversely, previous studies have demonstrated that local treatment with MFAT-PTX in a canine model is well tolerated, with no recorded toxicity or hypersensitivity [5]. Pharmacokinetic (PK) data from these studies indicated low drug presence in the circulatory system and a tendency for the drug to remain in extravascular compartments, resulting in low systemic toxicity but high local drug concentrations that enhance pharmacological efficacy against tumors [5]. The safety of MFAT has been well established in dogs treated for various conditions, including arthropathies, ulcers, and immune-mediated lesions [35]. In human medicine, MFAT has been safely used for orthopedic, vascular and inflammatory conditions, as well as pain management, with no major adverse effects recorded [43-47]. Moreover, intracranial fat application is commonly used in human surgery to cover skull base defects and fill spaces left by tumor removal, further supporting MFAT's potential safety and utility in brain tumor treatments.

In this study, we aimed to assess whether MFAT-PTX could be a viable treatment option for spontaneous canine gliomas, with possible implications for human therapy. The epidemiological data and clinical signs in our canine cohort, were consistent with existing literature on canine gliomas [22,48]. The cohort dimension (9 client-owned canine patients) resulted to be at least comparable in respect to similar studies [49,50]. Radiotherapy and surgical options were excluded based on the owners' preferences due to limited availability, high costs, and the risks associated with repeated anesthesia. Thus, our focus was on pharmacological treatment, which does not preclude future combined therapeutic approaches.

The safety of the MFAT-PTX treatment was the primary concern of this study. No significant short- or mid-term adverse effects were observed related to either the injection procedure or the drug delivery system. In contrast, the control group dogs treated with conventional chemotherapy (lomustine) experienced side effects, further highlighting the safety of MFAT-PTX.

The PK study confirmed that PTX's localization in the circulatory system was minimal, with the drug remaining primarily in extravascular compartments, which aligns with the observed low systemic toxicity in treated dogs. Interestingly, PK results demonstrated reproducibility within individual dogs, with significant residual amounts of PTX detected in tumors up to four months post-treatment, suggesting long-term efficacy.

In terms of clinical outcomes, the MFAT-PTX treatment protocol was straightforward and efficiently executed in all cases. Neuronavigation was used to ensure precise placement of MFAT-PTX directly into the tumor mass during biopsy. No surgical complications were reported and a synthetic, biocompatible prosthesis was used to cover the craniectomy site, facilitating postoperative assessments and potential re-treatments without the need for additional craniectomies.

The primary goal of brain tumor therapy is to enable patients to maintain a normal or acceptable quality of life (QoL). In our study,

no adverse effects related to the drug or procedure were observed, as confirmed by PK assessments. Neurological symptoms resolved completely in three out of six treated dogs, with seizures well-controlled in all but one case. Three dogs were reported by their owners to have an excellent QoL post-treatment. One dog was reported to have a poor QoL due to seizures, despite normal performance in other parameters, while another dog had an acceptable QoL, though it remained lethargic. Overall, the clinical response in three of the six treated dogs was very positive, with stable conditions observed in the remaining two. Tumor volume reductions paralleled clinical improvements, with a median overall survival of 188 days in treated dogs, compared to 177 days in the control group, where no complete remission was observed, and tumor volumes increased. These survival times are consistent with previous reports for dogs with gliomas [22,38,51].

Histological analysis revealed no signs of lesions or neurotoxicity in brain tissue outside the treated tumor site. All tumors were classified as grade II oligodendrogliomas, which, although coincidental, allowed for the evaluation of data from a homogeneous group, reducing potential biases. Notably, some dogs had initial biopsy diagnoses of different tumor types, which were later revised to grade II oligodendrogliomas post-mortem. This phenomenon has been observed in other studies [37]. The MFAT-PTX treatment induced a granulomatous inflammatory reaction and frequent infiltration of foamy macrophages, suggesting phagocytosis of the lipid-containing material. Necrotic areas around MFAT remnants indicated the local efficacy of the chemotherapy, with the treatment appearing to cause necrosis of neoplastic cells and promoting a reactive glial-mesenchymal response [52].

The mechanism behind MFAT-PTX's effect on brain tumor cells is complex and likely involves several factors. One potential mechanism is the inhibition of angiogenesis, a critical event in tumor growth, which PTX is known to impede. Additionally, MFAT may induce antitumor immunity, similar to endothelial vaccines that have been shown to reduce tumor growth in gliosarcoma models [53]. Future research directions include further studies in order to explore whether serum or CSF antibodies in treated patients could stain glioma micro vessels, supporting this immune response mechanism. The major limitation of this study is the low number of patients. Indeed, some dogs ended their life for reasons independent of the gliomas, shortening the follow-up period. A further investigation with a larger group of canine patients is therefore warranted to further confirm and expand our results.

Conclusions

This study represents the first drug delivery system trial in dogs with significant implications for human glioma treatment due to its strong translational potential. We prioritized patient safety, quality of life and procedural feasibility, with assessments conducted by both veterinarians and human surgeons. Our work demonstrated that local administration of micro fragmented adipose tissue loaded by paclitaxel (MFAT-PTX) in dogs with spontaneously occurring gliomas led to clinical improvement and Tumor volume reduction in most cases, without requiring Tumor removal. These findings suggest that MFAT-PTX could be a valuable adjuvant therapy alongside surgery and radiotherapy. The multidisciplinary approach and promising results of this study support further investigation in larger canine cohorts and provide a crucial proof of concept for a potential translational human application.

Author Contributions: Conceptualization, O.Z., G.A., A.P.; methodology, O.Z., E.G., L.P., C.D., M.P.D., C.T., M.M., D.Z., M.K., C.C., A.P., S.B., L.F., L.M., S.N., R.P., G.A., F.P., M.D.C., F.R., M.S., E.M., A.P; formal analysis, O.Z., E.G., R.P., G.A., A.P; investigation, O.Z., E.G., L.P., C.D., M.P.D., C.T., M.M., D.Z., M.K., C.C., A.P., S.B., L.F., L.M., S.N., R.P., G.A., F.P., M.D.C., F.R., M.S., E.M., A.P .; resources, O.Z.; data curation, O.Z., G.A., A.P.; writing—original draft preparation, O.Z., E.G., F.R., E.M., G.A., A.P.; writing—review and editing, O.Z., F.R., E.M., G.A., A.P. visualization, O.Z.; supervision, O.Z., G.A., A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: No similar manuscript is or will be under consideration for publication elsewhere. All data underlying the research presented in the manuscript is stored in the San Michele Veterinary Hospital and is publically available upon request at any time. All animal procedures were performed in accordance with the European code of good veterinary practice and following the guidelines defined by the Italian Presidency of the Council of Ministers and published by the General Directory of Animal Health and Veterinary drugs of the Italian Ministry of Health (147/CSR, 277). The owners of the dogs selected for the treatment were thoroughly informed about the entire procedure and signed a formal agreement with the San Michele Veterinary Hospital in acceptance of the treatment. Owners have also accepted that their dogs will undergo post-mortem examination at the end of their life.

Acknowledgments: We wish to thank Dr. Daniele Spaziante and Dr. Domenico Fugazzotto for their collaboration and Dr. Alessandro Rotilio for his assistance with the synthetic implant.

Conflicts of Interest: Offer Zeira and Giulio Alessandri are scientific advisors of Lipogems International. The authors have no additional financial interests.

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