Chronic suppressive therapy with calcium channel antagonists for refractory meningiomas

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In this article, the authors review the research supporting the use of calcium channel antagonists (CCAs) in the treatment of recurrent or unresectable meningiomas. Calcium channel antagonists (for example, diltiazem and verapamil) are known to augment the effects of chemotherapy drugs (for example, vincristine) in multiple cancers. Although it was initially thought that this occurred by interference with calcium-dependent secondary messenger systems, it appears that other mechanisms account for this effect. The authors’ initial work in this field was based on the then-emerging data that meningiomas are receptor positive for growth factor receptors (for example, platelet-derived growth factor [PDGF]), which are known to trigger calcium-dependent secondary messenger pathways. In fact, they were able to show that CCAs block the growth stimulatory effects of multiple growth factors, including PDGF, in vitro and augment the growth inhibitory effects of hydroxyurea and RU486 (mifepristone). The authors have shown similar in vivo growth inhibition by these agents. In addition, diltiazem- and verapamil-treated meningiomas are less vascular and smaller, with decreased cell proliferation and increased apoptosis. The use of CCAs is attractive as an adjunct treatment for unresectable or recurrent meningiomas because they are safe drugs with well-known side effect profiles that lend themselves to long-term chronic therapy. (DOI: 10.3171/FOC-07/10/E10)

KEY WORDS • calcium antagonist • calcium channel • chronic therapy • hydroxyurea • meningioma • verapamil

Complete resection is the treatment of choice for intracranial meningiomas but may not be possible when the tumor invades critical structures such as the cavernous sinus or sagittal sinus. This is confounded even more by the fact that up to 20% of meningiomas exhibit an aggressive phenotype that does not respond to standard therapies.10 Thus, adjuvant therapies are critical for patients with this subset of meningiomas. Radiation therapy and stereotactic radiosurgery are good adjuvant therapies but are limited by radiation neurotoxicity, tumor size constraints, and injury to adjacent vascular or cranial nerves.8,9 Standard chemotherapeutic treatments have been disappointing.13 Even drugs like temozolomide that have shown efficacy against malignant brain tumors have failed to inhibit the growth of refractory meningiomas in a Phase II study.4 Therefore, researchers continue to investigate other options to improve the results obtained from these treatments.

Our interest in using voltage-dependent CCAs such as diltiazem, verapamil, and nifedipine for the treatment of human intracranial meningiomas stems from observations that these are immunohistochemically positive for several growth factor receptors, such as PDGF, EGF, FGF, and vascular endothelial growth factor. These growth factors are known to trigger calcium-dependent secondary pathway systems with potent mitogenic effects when stimulated in vitro.1,2,5,7,17,20,29,30 We initially hypothesized that interruption of these signal transduction pathways could result in inhibition of meningioma cell growth. As our work progressed, it became clear that these drugs most likely worked through a mechanism that did not involve intracellular calcium signaling.12,13 This is based on our observations that most meningioma cells do not have surface L-type calcium channels (the target of the CCAs used in our studies). Furthermore, although growth factor stimulation of meningiomas using EGF and PDGF results in increased intracellular calcium, pretreatment with the CCAs diltiazem and verapamil did not consistently alter this signal transduction pathway.13

Abbreviations used in their article: CCA = calcium channel antagonist; EGF = endothelial growth factor; FDG = 18F-2-fluoro-deoxy-D-glucose; FGF = fibroblast growth factor; FLT = 18F-2’-fluoro-2’-deoxy-3’-deoxy-l-thymidine; MR = magnetic resonance; PET = positron emission tomography; PDGF = platelet-derived growth factor.
In a more general sense, the idea of using CCAs for the treatment of cancer is supported by a large body of literature showing that use of CCAs either alone or in combination with chemotherapy drugs (for example, vincristine and adriamycin) results in enhanced cytotoxicity in multiple cell lines (for example glioma, prostate, and lung).

This is significant because, in contrast with most treatment regimens, CCAs are safe drugs with well-known side-effect profiles that lend themselves to long-term chronic therapy. This concept has also gained acceptance in the treatment of patients with other diseases, such as human immunodeficiency virus–infected individuals in whom current treatment algorithms convert a quickly fatal disease into a chronic disease. In the case of the relatively slow-growing meningioma, long-term therapy may be necessary for tumors that have proven refractory to complete resection and radiation treatment or that have recurred despite aggressive therapy.

Calcium channel antagonists have been demonstrated to induce apoptosis, decrease cellular proliferation indices, and increase metastatic potential and invasion in many cancer cell lines. In the majority of these studies, the authors were unable to demonstrate whether this effect was due to inhibiting the calcium-dependent secondary messenger systems within these cells. The enhanced cytotoxicity exhibited when CCAs are added to chemotherapies is attributed to the blocking of the multidrug resistance protein P-glycoprotein. P-glycoprotein acts as an adenosine triphosphate–dependent drug efflux pump, reducing the intracellular accumulation of chemotherapeutic drugs. Other possible mechanisms of growth inhibition by CCAs include interference with the action of protein kinase C, calmodulin, phosphodiesterase, or the c-ras oncogene guanosine triphosphate–binding protein.

The CCAs diltiazem, verapamil, and nifedipine have been studied in meningioma cell culture and in a mouse xenograft model, as described later. In these studies, the meningioma cells have shown decreased growth when the CCAs are used alone or in conjunction with hydroxyurea or mifepristone (RU486). In vitro, CCAs block the mito-
genic effects of various growth factors, block fetal calf serum–induced intracellular mobilization, induce a G1 cell cycle arrest, and augment the growth inhibitory effects of both hydroxyurea and RU486.13–16,26 In vivo, xenograft meningioma mouse tumors are smaller and show evidence of decreased cellular proliferation, decreased vascular density, and increased apoptosis.16,26

**Laboratory Studies**

**Calcium Channel Antagonists as Single Agents**

Calcium channel antagonists inhibit the growth of meningiomas grown in vitro in a dose-dependent manner both alone and after the cells are exposed to the growth stimulatory effects of multiple growth factors including fetal calf serum, EGF, PDGF, FGF, and insulin-like growth factor–1.13–15 A dose-dependent decrease in cell growth was seen when verapamil, nifedipine, or diltiazem was added to serum-containing media.13–15 The use of the intracellular calcium-chelating fluorescent dye Fura-2 showed that the addition of fetal calf serum and various growth factors to meningioma cells in culture resulted in calcium fluxes that could be blocked with the addition of verapamil.15 This suggested that the calcium-dependent signaling pathways may be altered with the addition of a CCA.15 This was not always a consistent finding, however, leading to questions about the mechanism by which CCAs inhibit in vitro meningioma growth. Despite this uncertainty, the promising results of these studies led us to proceed with in vivo testing to determine the effects of CCAs on xenograft meningioma growth.

We used a meningioma mouse xenograft flank model to study the effects of CCAs in an in vivo model. In our first study, meningioma xenograft-implanted mice were given ad libitum access to CCAs added to drinking water. Diltiazem and verapamil treatment resulted in dose-dependent decreased tumor growth over time compared with tumor growth in animals in the control groups.16 Drug treatment was not curative, however, indicating a growth-suppressive effect of CCAs.16 Mouse serum drug levels increased with increasing doses of drug in the drinking water.16 Unfortunately, this effect was not consistent, and the growth inhibition of CCAs alone was modest at best. This led us to an interest in using the CCAs in combination with chemotherapeutic agents to find a more effective treatment regimen.

![Fig. 2. A. Photomicrographs. Immunohistochemical staining for MIB-1 and factor VIII, as well as terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining for apoptosis on IOMM-Lee flank tumors. Note the decrease in immunohistochemical staining for MIB-1 and factor VIII (arrows) in the diltiazem and combined hydroxyurea/diltiazem groups, as well as the increase in number of apoptotic cells with treatment (arrow denotes brown-stained apoptotic cell). Original magnification × 40; bar = 25 μm. Reprinted with permission from Ragel et al., 2006.](../image)
Calcium Channel Antagonists and Chemotherapeutic Combination Therapy

Clinical trials for treatment of meningiomas using hydroxyurea and RU486 reported in the neurosurgical oncology literature have been generally disappointing. Although the drugs are well tolerated in most patients, the results have not been consistent or durable. Given the earlier discussion, it is natural to propose that adding a CCA to the relatively weak activity of hydroxyurea or RU486 might result in increased meningioma cell growth inhibition compared with the use of either agent alone. In fact, the addition of diltiazem or verapamil to hydroxyurea or RU486 intensified in vitro meningioma growth inhibition by 20 to 60% by inducing apoptosis and G1 cell cycle arrest. It was unclear by which mechanism diltiazem and verapamil exert their effect on meningiomas, but the growth inhibitory effects of CCAs appeared to be in addition to the growth inhibitory effects of either hydroxyurea or RU486. With these more promising results, we thought that moving into an animal model would cast further light on the feasibility of this combination therapy.

Using the same mouse model as described earlier, we were able to demonstrate a dose-dependent decrease in tumor sizes with the combination CCA and hydroxyurea or RU486 treatment regimen (Fig. 1). This combination therapy appeared to be additive for the effects of each drug (that is, tumor reduction was the sum of reduction associated with treatment with each drug used alone). Meningioma flank tumors treated with combinations of drugs were smaller, and immunohistochemical analysis of the malignant meningioma cell line IOMM-Lee tumors showed a 10% decrease in the MIB-1 ratio (0.41 to 0.30), an approximately 75% decrease in microvascular density, and an increase in the number of cells undergoing apoptosis (Figs. 2 and 3). We have repeated these experiments in an intracranial meningioma model using xenograft meningioma cells genetically altered to express luciferase activity that allows us to measure tumor growth noninvasively and without the cost and difficulty of MR imaging (unpublished data). Given the positive results of our in vitro and in vivo experiments of combination therapy, the next step is testing in human clinical trials for safety and eventually for efficacy.

Proposed Human Clinical Trial

Because of our success in laboratory studies in which we have combined hydroxyurea and various CCAs, we have designed an upcoming clinical trial with primary objectives to determine the safety of the combination of hydroxyurea and verapamil for treatment of patients with progressive, recurrent meningiomas by characterizing the toxicity of this drug combination. In addition, our goal is to determine the objective response rate of patients treated with this drug combination by MR imaging and metabolic PET imaging. We also plan to determine the 6- and 12-month progression-free survival rates of the treatment population.

Our selection of hydroxyurea and verapamil for the clinical trial is based on the positive results obtained in our studies and, in particular, on the clinical profiles of these drugs, which are both commonly used for treatment of numerous conditions. Hydroxyurea is well absorbed after

FIG. 3. Bar graphs depicting proliferative indices (MIB-1 ratio) (A), microvascular density (factor VIII) (B), and apoptotic cells (TUNEL) (C) in IOMM-Lee meningioma mouse flank tumors. Mice were allowed to drink water with vehicle control (0.1% ethanol), diltiazem 50 mg/kg, or a combination of diltiazem 50 mg/kg and hydroxyurea 100 mg/kg ad libitum. Animals were killed by Day 56. Error bars represent standard deviation of nine measurements (three mice per group, one slide per mouse, three measurements per slide). Overall, both the proliferative indices (MIB-1) and vascular density (factor VIII) of the tumors treated with diltiazem alone or the combination diltiazem/hydroxyurea were less than control animals. The number of apoptotic cells increased with treatment. A statistically significant difference was noted between the control versus diltiazem/hydroxyurea group for proliferative indices, microvascular density, and apoptosis. *p < 0.05, analysis, post hoc Tukey. Reprinted with permission from Ragel et al., 2006.
Calcium channel antagonists for meningiomas

oral administration, with peak serum concentration achieved in 2 hours. Serious adverse reactions associated with hydroxyurea include myelosuppression, with leukopenia more prominent than thrombocytopenia. In addition, other less serious side effects have also been noted. Verapamil is commonly used for the treatment of angina, hypertension, supraventricular arrhythmias, and migraine prophylaxis. Verapamil is contraindicated in patients with left ventricular dysfunction, second- or third-degree atrioventricular block, or sick sinus syndrome. Serious adverse reactions associated with verapamil use are congestive heart failure, hypotension, and bradycardia.

Study Design

Our initial study will be a Phase II design with an interim analysis (Phase I) to assess for toxicity of the combination of these two drugs. No dose escalation will be performed because authors of numerous studies have established the dosage of hydroxyurea for the treatment of meningioma. Verapamil also has a long clinical history, and dosing will be limited by bradycardia and hypotension, making dose escalation infeasible. We have calculated the number of patients we will treat at our institution in this initial study and assume an 80% power with an alpha value equal to 0.05 significance level, a null hypothesis response rate of 20% (derived from prior clinical trials of hydroxyurea), and an alternative hypothesis response rate of 50%. In our two-stage design, we will examine results after the first eight patients (Stage 1); if there are fewer than two responses, the trial will be halted as ineffective. If there are at least two responses, 10 more patients will be enrolled (Stage 2). If there are fewer than six responses out of all 18 patients, the study drug combination will be deemed ineffective. Safety analysis will be performed between Stages I and II.

Study Procedures

Patients with histologically confirmed meningioma of any World Health Organization grade, with imaging demonstration of at least 25% increase in tumor cross-sectional area measured on computed tomography/MR imaging within the last 6 months who refuse or are unable to tolerate surgical or radiation therapy options will be included in our study. Because of the concerns of myelosuppression in patients receiving hydroxyurea, only patients with white blood cell and platelet counts above minimal levels will be included.

Patients will undergo preliminary MR imaging of the brain with and without Gd contrast to assess tumor measurements within 1 week of beginning treatment. They will also undergo PET with metabolic (or FDG) and proliferation (or FLT) markers to assess tumor activity. This will provide a baseline for each patient to allow assessments of tumor growth during the study. Patients will then undergo MR imaging with Gd (or computed tomography with contrast) and PET imaging every 3 months. Our rationale for the PET imaging is based on the possibility of gaining earlier indications of treatment efficacy. In the case of relatively slow-growing meningiomas, significant changes often take up to 1 or 2 years to determine the results of a given treatment. It is hoped that using PET imaging, especially that of the FLT-measured proliferation rate, we might be able to determine the efficacy of our combined treatment regimen after 3 to 6 months. Nevertheless, MR imaging is the gold standard for determining tumor growth and will be used to identify progression of disease, which will be defined as a greater than 25% increase of largest cross-sectional area by two orthogonal measurements on MR images. Patients will be monitored for up to 2 years on the study.

In this Phase I/II study, safety and toxicity will be our main objectives. Major concerns are hypotension and bradycardia in elderly patients and myelosuppression in all patients. If we can demonstrate that the combination of hydroxyurea and verapamil is well tolerated with a minimal side-effect profile, we will then focus on efficacy in a Phase III trial. We will be looking for some indication of efficacy in our initial Phase II study as well. We also will determine the utility of FLT and FDG PET in assessing the response rate of this combination therapy. If helpful, we would include these assessments in future studies. A Phase III study would, of course, have to be multiinstitutional to obtain patient numbers necessary to prove or disprove efficacy. The study power calculations would be based on the limited efficacy data from the trial proposed earlier. For this Phase III trial we might compare meningioma growth response to verapamil alone with the response to the combination therapy, which could help in determining the relative contributions of each of the drugs used in this drug combination therapy. More importantly, it could shed light on the efficacy of using verapamil alone for treatment of refractory meningiomas. This would be important for the concept of chronic suppressive therapy because it would be supposed that verapamil alone would be much better tolerated than long-term hydroxyurea treatment. One might envision using the combination therapy to decrease tumor size and then switching to single-agent verapamil therapy for chronic suppression of regrowth of a given meningioma. Much work remains to be done, but this therapeutic approach certainly has both appeal and promise.

Conclusions

Calcium channel antagonists can block the stimulatory effects of numerous growth factors on meningioma cell culture and augment the growth inhibitory effects of the chemotherapy drugs hydroxyurea and RU486. They have also been shown to increase the effectiveness of hydroxyurea and RU486 in a xenograft mouse meningioma tumor model with evidence of decreased cellular proliferation, decreased vascular density, and increased apoptosis. Although the mechanism of action is unknown, these drugs might disrupt intracellular calcium homeostasis or interfere with key elements of the growth factor signal transduction pathways. These studies suggest a possible role for CCAs in adjuvant therapy for recurrent or unresectable meningiomas when used in combination with other treatments. The results of our clinical trial to investigate the safety and preliminary efficacy of the combination of CCAs with chemotherapy drugs might ultimately allow the transition of this theoretical application to the clinical setting.

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References


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