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Ion-channel blockers and glioblastoma risk and outcome: A nested case-control and retrospective cohort studies

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Abstract

Purpose—Mutations in ion-channels are common among patients with glioblastoma multiforme (GBM) and promote cell migration and invasion. We sought to evaluate the association between the use of specific ion-channel blockers such as digoxin, amiodarone, diltiazem and verapamil and GBM risk and survival.

Methods—We conducted a nested case-control study in a large primary care database from the UK. Cases were defined as all individuals with incident diagnosis of GBM during follow-up. For each case, up to four controls were selected using incidence-density sampling. The primary exposure of interest was active treatment with each of the four ion-channel blockers. We used conditional logistic regression to estimate odds-ratios (ORs) and 95% confidence-interval (CI) for the association between ion-channel blocker use and GBM risk. We then performed a Cox regression analysis among those diagnosed with GBM in order to evaluate the association between use of ion-channel blockers and overall survival. Both analyses were adjusted to common confounders.

Results—The study included 1,076 cases and 4,253 matched controls. There was no statistically significant difference between cases and controls in cardiac and metabolic risk factors. There was no change in GBM risk in active users of ion-channel blockers compared to non-users. Among patients with GBM, active users of amiodarone had worse survival compared to never users with an HR of 4.41 (95%CI 1.95-9.96). There was no statistically significant change in survival among diltiazem, verapamil or digoxin users.

Conclusion—Treatment with specific ion-channel blockers was not associated with the risk of GBM, but was associated with worse survival in GBM patients.

Keywords

GBM; risk; survival; digoxin; amiodarone; diltiazem; verapamil

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Introduction

Glioblastoma multiforme (GBM) is the most common and lethal type of primary brain tumor with approximately 15,000 cancer deaths annually in the US and an incidence of 2-3 cases per 100,000 person years in North America and Europe (1,2). The disease originates from glial cells in the brain and spinal cord and presents with rapid growth and invasive phenotype. The prognosis of GBM is dismal with a median survival of 15 months (3,4). Risk factors are largely unknown, with the exception of radiation exposure and rare genetic syndromes including neurofibromatosis, tuberous sclerosis and Li-Fraumeni syndrome (5).

Several preclinical studies suggest an important role for sodium (6-9), potassium (Ca activated (10-19), outward rectifying (20,21), and hERG (22,23)) and chloride ion-channels (24-28) as well as Na-K-Cl cotransporter (29,30) on GBM cell migration and parenchymal invasion, possibly through effects on cellular volume and shape. Mutations in at least one of the ion-channel genes are detected in up to 90% of patients with GBM, a finding associated with worse survival (31). Other studies found reduced cell growth and increased apoptosis in glioma cell lines treated with medications that inhibit specific ion-channels, such as digoxin (blocker of the sodium potassium ATPase) (32), amiodarone (potassium channel blocker) (33), diltiazem, and verapamil (calcium channel blockers) (34,35).

Thus, it is conceivable that ion-channel blockers may influence both GBM risk and outcome. To date, there are no epidemiological studies that have investigated the association between the use of ion-channel blockers and GBM risk or survival. We sought to evaluate this association in a large population representative general practice database from the United Kingdom (UK).

Methods

We conducted a nested case-control study to examine the association between ion-channel blocker use and the risk of GBM and a retrospective cohort study to determine the effect of these medications on GBM survival. The study was approved by the Institutional Review Board at the University of Pennsylvania and by the Scientific Review Committee of THIN.

Data source

The Health Improvement Network (THIN), is a large electronic medical record database from the UK that contains comprehensive medical records of over 10 million individuals treated by general practitioners. THIN was established for research purposes, and its population as a whole was shown to be representative of the entire UK population (36). All practices contributing data to THIN follow a standardized protocol of entering and transmitting information to the central database. Data quality is monitored through routine analysis of the entered data (37,38). Cancer incidence in THIN was previously shown to be comparable to that in the entire UK population as reported in cancer registry data (39).

Nested case control study

Study Design and population—In order to evaluate GBM risk associated with ion channel blockers use we conducted a nested case-control study with incidence density sampling among individuals receiving medical care from a THIN practitioner between 1995-2013 (40).

Observation period—Follow-up time for the case-control study started at the later of either the date when the THIN practice started using the electronic medical record software or 6 months after the date at which the patient registered with the clinic (41). Follow-up time ended on the GBM diagnosis date for cases (index date) and on the same assigned date for the matched controls.

Case selection—Individuals with at least one medical Read code (the standard primary care classification system in the UK) for GBM during follow-up (BBbL.11) were defined as Cases. The first date of GBM diagnosis in the electronic medical record was defined as the Index date. We excluded individuals with diagnosis during the first 6 months of follow-up in order to include only incidence cases (a total of 235 prevalent GBM cases were excluded) (41).

Selection of controls—Controls were selected using incidence density sampling (40). Eligible controls for each case comprised of all individuals without a diagnosis of GBM at the date the case was diagnosed. Up to four eligible controls were matched for each case based on age at index-date, sex, practice site, and both duration and calendar time of follow-up. All controls matched with a case were assigned the same index date as the case.

Retrospective cohort study

Study Design and population—In order to evaluate the association between ion-channel blockers use and survival among GBM patients, we conducted a retrospective cohort study. The cohort was of the 1,076 patients with GBM diagnosis (‘cases’ from the case-control study).

Observation period—Follow-up started on the date of cancer diagnosis and censoring was made on earliest of date of death, transferring out of the database, or reaching the end date of the database.

Exposures and Covariates—The primary exposure of interest for both studies was treatment with one of four ion-channel blockers: diltiazem and verapamil (calcium channel blockers), amiodarone (potassium channel blocker) and digoxin (blocker of the sodium potassium ATPase). Users of medication were defined as active users, long-term users, and former users for both studies. Active users were defined as individuals with their last prescription within 6 months prior to GBM diagnosis. Long-term users were defined as individuals with their first prescription more than one year before GBM diagnosis and last prescription within 6 months prior to diagnosis. Former users were defined as individuals with only one prescription for the specific medication given more than six months before GBM diagnosis. Although there are no known variables that are associated with both GBM

diagnosis and prescription of ion-channel blockers, we adjusted all analyses to the following potential confounders: obesity defined as BMI above 30kg/m^2, ever smoking, and past medical history of diabetes or cardiovascular disease (according to diagnostic codes).

Statistical Analysis—In the nested case-control study, baseline characteristics of GBM cases and controls were compared using univariate conditional logistic regression. Conditional logistic regression was also used to estimate odds ratios (ORs) and 95% confidence interval (CI) for the associations between active use, long-term use, and former use of each ion-channel blocker and GBM risk, compared to never users. This analysis was adjusted for obesity (BMI>30), ever smoking, diabetes and cardiovascular disease. In addition, we repeated the analysis using former users as a comparison group in order to reduce possible confounding by indication (former users are expected to have similar indication for therapy as active users). In the cohort study, a Cox regression analysis was performed, after testing for the proportionality assumption, among GBM patients to evaluate the hazards ration (HRs) and 95%CI of the association between use of each ion-channel blocker and survival after GBM diagnosis. This analysis was adjusted for age, sex and duration of follow-up before GBM diagnosis, in addition to obesity, ever smoking, diabetes and cardiovascular disease. All analyses were performed using STATA 13.

Results

The study population included 1,076 patients with GBM and 4,253 matched controls. The mean age of cases and controls was 62.6 years (±12.0 SD). Among participants, 59.3% (3,160) were males and 40.7% (2,169) females. The mean duration of follow-up was 6.1 years (±4.0 SD). There was no statistically significant difference between cases and controls in cardiac and metabolic risk factors, such as obesity (22.5% vs. 19.8%), ever smoking (45.8% vs. 42.6%), diabetes (7.8% vs. 9.1%) and cardiovascular disease (8.2% vs. 8.5%) (Table 1).

In the case control study, compared to non-users of ion-channel blockers, neither active users nor long-term users were associated with an increased risk of GBM (Table 2). When former users were used as the reference group, there was a non-significant higher risk among active users of amiodarone (OR 3.03, 95%CI 0.89-10.33) (Table 3).

The cohort study was of the 1,076 patients with GBM and had 1925 person years of follow-up after the cancer diagnosis. The incidence rate of death was 397 per 1000 person years. Both any active users and long term users of amiodarone had worse survival compared to non-users with HRs of 4.41 (95%CI 1.95-9.96) and 6.94 (95%CI 2.56-18.81), respectively. The median survival among active amiodarone users was 66.5 days (IQR 28-78) compared to 230.5 days (89-564) in non-users (Table 4, 5).

Discussion

The current large nested case-control study demonstrated no association between use of diltiazem, verapamil, amiodarone or digoxin and GBM risk. However, among patients diagnosed with GBM, active users of amiodarone had shorter survival compared to non-

users (HR 4.41, 95%CI 1.95-9.96). The effect increased among long term users with duration of therapy of more than one year (HR 6.94, 95%CI 2.56-18.81).

These results are in accordance with previous genetic studies showing high prevalence of mutations in ion-channel genes in tissue samples from patients with GBM (31). However, other studies using in-vitro glioma cell lines suggested enhanced sensitivity of glioma cells to chemotherapy following treatment with digoxin or amiodarone (32,33). Among the biological mechanisms proposed for this protective effect were inhibition of tumor invasion and migration through effects on cellular volume and shape; induction of apoptosis through cellular hyperpolarization; and reduced secretion of the tumor promoting vascular endothelial growth factor (VEGF). One possible explanation for the inconsistent results reported in the literature is that established glioma cell lines poorly represent primary GBM tumors as there are significant genomic and transcriptome differences between the two (42). Because such changes can dramatically impact a cell's responsiveness to physiologic and pharmacologic stimuli, it remains unclear whether primary GBMs also exhibit this enhanced sensitivity. In addition, the variability in channel properties may also underlie this difference. For example, while most calcium channel blockers target L-type calcium channels, most of the protective effect in cancer patients is mediated through T-type calcium channels.

The current study is the first epidemiologic study in humans to test the effect of specific ion-channel blockers in GBM patients. THIN is a large population representative database (36) that contain detailed information regarding all prescriptions provided by the general practitioner as well as specific cancer diagnosis. The mean duration of follow-up for cases and controls in THIN is more than six years, allowing the assessment of medication's effect at different time periods during tumorigenesis. The completeness of prescription histories, cancer diagnosis and death data was shown in previous studies in THIN (37,39,43). By analyzing prescriptions more than one years before GBM diagnosis we were able to evaluate for reverse causality as a possible explanation for the observed association (the possibility that undiagnosed cancer prompted medical evaluation that lead to the diagnoses of other medical conditions). We were also able to exclude patients with GBM in the first six months after registration to a clinic, in order to avoid prevalent cases.

A risk for possible preferential detection bias as a result of medical evaluation in patients treated with ion-channel blockers (i.e. brain imaging in patients after syncope), was probably low, due to the rapid and aggressive behavior of GBM and its early manifestation of symptoms. The possibility of confounding by indication was excluded by observing similar results as the primary analysis when using former medication users as the reference group.

The main limitations of the study included use of medical diagnostic codes for GBM rather than pathology reports. Additionally, THIN does not contain information on cancer stage or histology. Although we used codes specific for GBM it is possible that patients with other types of glioma were included. However, we expect any such misclassification to be rare. Of note, previous studies demonstrated similar incidence of solid tumors in THIN compared to UK cancer registry data (39). Furthermore, we lacked information regarding familial cancer syndromes or previous radiotherapy that might increase GBM risk. Due to lack of information regarding anti-GBM treatment we were not able to rule out possible interaction

between ion-channel blockers and chemotherapy. Additionally, we relied on prescription information to determine medication use, which may lead to misclassification. However, we expect the medication use data to be more accurate among long-term users who would need to repeatedly obtain prescription from their general practitioners on a monthly or bi-monthly basis. The fact that we observed similar findings associated with active use and long-term use would seem to suggest such misclassification was less likely. Of note, since these medications (mainly amiodarone and digoxin) have a narrow therapeutic window and there is not much variability in the dose prescribed a dose response analysis was not performed. The small number of patients within each treatment group was an expected limitation considering the known incidence of GBM and the indications for treatment with ion-channel blockers. Finally, we did not have information regarding the specific cause of death of patients, however, because of the highly aggressive nature of GBM almost all patient die of the disease.

In summary, we demonstrated decreased survival among users of amiodarone diagnosed with GBM. The effect increased with longer duration of therapy. There was no change in GBM risk both in any users and long term users of amiodarone. Future epidemiological studies should validate these findings in additional large databases and evaluate the possible biological mechanism behind the association. Furthermore, it is not known whether other novel anti-arrhythmic medications have similar effects on GBM outcome as amiodarone and whether they might be better treatment options in those patients. Thus, no change in clinical recommendations regarding the use of amiodarone in GBM patients can be made at this point.

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Key points

- Mutations in ion-channels are common among patients with GBM and promote cell migration and invasion.
- We evaluated in a large population representative database the association between use of digoxin, amiodarone, diltiazem or verapamil and GBM risk and survival.
- The use of specific ion-channel blockers did not change GBM risk.
- Patients diagnosed with GBM who were actively taking amiodarone had worse survival.

Table 1

Characteristics of cases and controls

Variable	Cases (N=1,076)	Controls (N=4,253)	P-value
Age (Mean ± SD)	62.8 (12.0)	62.6 (12.0)	NA
Male sex (N, %)	639 (59.4)	2,521 (59.3)	NA
Duration of follow-up (Mean ± SD)	6.1 (4.0)	6.1 (4.0)	NA
Obesity (N, %)	242 (22.5)	842 (19.8)	0.05
Ever Smoking (N, %)	493 (45.8)	1,811 (42.6)	0.05
Diabetes mellitus (N,%)	84 (7.8)	387 (9.1)	0.19
Cardiovascular disease (N, %)	88 (8.2)	362 (8.5)	0.59
Medications*			
Diltiazem:			
Active users (N, %)	19 (1.8)	100 (2.4)	0.26
Long term users (N, %)	16 (1.5)	89 (2.1)	0.23
Verapamil:			
Active users (N, %)	6 (0.6)	23 (0.5)	0.93
Long term users (N, %)	4 (0.4)	20 (0.5)	0.68
Amiodarone:			
Active users (N, %)	6 (0.6)	16 (0.4)	0.4
Long term users (N, %)	4 (0.4)	13 (0.3)	0.72
Digoxin:			
Active users (N, %)	11 (1.0)	53 (1.3)	0.49
Long term users (N, %)	10 (0.9)	48 (1.1)	0.51

*Active users were defined as individuals with their last prescription within 6 months prior to GBM diagnosis. Long-term users were defined as individuals with their first prescription more than one year before GBM diagnosis and last prescription within 6 months prior to diagnosis.

Table 2

GBM risk among active long term users* of specific ion-channel blockers compared to never users

Medication	Diltiazem	Verapamil	Amiodarone	Digoxin
Non-users	Ref.	Ref.	Ref.	Ref.
Active users				
Unadjusted OR (95%CI)	0.75 (0.46-1.23)	1.04 (0.42-2.56)	1.50 (0.59-3.83)	0.80 (0.41-1.53)
Adjusted OR† (95%CI)	0.76 (0.46-1.25)	1.02 (0.42-2.53)	1.45 (0.56-3.73)	0.79 (0.41-1.53)
Long-term users				
Unadjusted OR (95%CI)	0.72 (0.42-1.23)	0.80 (0.27-2.34)	1.23 (0.40-3.77)	0.79 (0.40-1.58)
Adjusted OR† (95%CI)	0.72 (0.42-1.24)	0.80 (0.27-2.34)	1.21 (0.39-3.73)	0.80 (0.40-1.59)

*Active users were defined as individuals with their last prescription within 6 months prior to GBM diagnosis. Long-term users were defined as individuals with their first prescription more than one year before GBM diagnosis and last prescription within 6 months prior to diagnosis.

†ORs adjusted for obesity (BMI>30), ever smoking, diabetes and cardiovascular disease. All variables were measured before index date.

Table 3

GBM risk among active and long term users of ion-channel blockers compared to former users*

Medication	Diltiazem	Verapamil	Amiodarone	Digoxin
Former users	Ref.	Ref.	Ref.	Ref.
Active users				
Unadjusted OR (95%CI)	1.02 (0.53-1.97)	1.99 (0.58-6.85)	3.03 (0.89-10.33)	1.38 (0.35-5.42)
Adjusted OR† (95%CI)	1.02 (0.53-1.98)	2.04 (0.59-7.02)	2.97 (0.87-10.17)	1.35 (0.34-5.32)
Long-term users				
Unadjusted OR (95%CI)	0.93 (0.48-1.82)	1.22 (0.33-4.49)	2.04 (0.54-7.70)	1.28 (0.37-4.46)
Adjusted OR† (95%CI)	0.92 (0.47-1.81)	1.27 (0.34-4.68)	2.04 (0.54-7.74)	1.29 (0.37-4.52)

*Active users were defined as individuals with their last prescription within 6 months prior to GBM diagnosis. Long-term users were defined as individuals with their first prescription more than one year before GBM diagnosis and last prescription within 6 months prior to diagnosis. Former users were defined as individuals with only one prescription for the specific medication given more than six months before GBM diagnosis.

†ORs adjusted for obesity (BMI>30), ever smoking, diabetes and cardiovascular disease. All variables were measured before index date.

Table 4

Survival analysis among patients with GBM comparing active users* of specific ion-channel blockers to non-users

Medication	Diltiazem	Verapamil	Amiodarone	Digoxin
Death, users				
Percent	14/19 (73.7)	5/6 (83.3)	6/6 (100.0)	9/11 (81.8)
Incidence rate per 1000 py	448.8	484.5	5544.3	895.3
Death, non-users				
Percent	750/1,057 (71.0)	759/1,070 (70.9)	758/1,070 (70.8)	755/1,065 (70.9)
Incidence rate per 1000 py	396.1	396.5	394.1	394.3
Unadjusted HR (95%CI)	1.15 (0.68-1.95)	1.85 (0.77-4.47)	5.56 (2.48-12.49)	1.99 (1.03-3.84)
Adjusted HR§ (95% CI)	0.82 (0.46-1.45)	1.66 (0.68-4.06)	4.41 (1.95-9.96)	1.56 (0.80-3.04)

*Active users were defined as individuals with their last prescription within 6 months prior to GBM diagnosis.

§HRs adjusted for age, sex, duration of follow-up before GBM diagnosis, obesity (BMI>30), ever smoking, diabetes and cardiovascular disease. All variables were measured before index date.

Table 5

Survival analysis among patients with GBM comparing long term users* of specific ion-channel blockers to non-users

Medication	Diltiazem	Verapamil	Amiodarone	Digoxin
Death, users				
Percent	12/16 (75.0)	3/4 (75.0)	4/4 (100.0)	8/10 (80.0)
Incidence rate per 1000 py	559.9	310.1	7807.5	823.9
Death, non-users				
Percent	750/1,057 (71.0)	759/1,070 (70.9)	758/1,070 (70.8)	755/1,065 (70.9)
Incidence rate per 1000 py	396.1	396.5	394.1	394.3
Unadjusted HR (95%CI)	1.13 (0.64-1.98)	1.45 (0.47-4.51)	8.41 (3.12-22.66)	1.93 (0.96-3.87)
Adjusted HR§ (95% CI)	0.81 (0.44-1.50)	1.27 (0.40-4.02)	6.94 (2.56-18.81)	1.50 (0.74-3.04)

*Long-term users were defined as individuals with their first prescription more than one year before GBM diagnosis and last prescription within 6 months prior to diagnosis.

§HRs adjusted for age, sex, duration of follow-up before GBM diagnosis, obesity (BMI>30), ever smoking, diabetes and cardiovascular disease. All variables were measured before index date.