Severe sustained cholestatic hepatitis following temozolomide in a patient with glioblastoma multiforme: case study and review of data from the FDA adverse event reporting system

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Glioblastoma multiforme (GBM) is the most frequent malignant brain tumor in adults. Its established first-line adjuvant treatment is radiotherapy in combination with temozolomide (TZM). Hematotoxicity is listed as a frequent adverse drug reaction in the US prescribing information and hepatotoxicity has been reported infrequently in the postmarketing period. We here present the case of a patient diagnosed with GBM who developed severe sustained cholestatic hepatitis following treatment with TZM. The cholestasis was not reversible after withdrawal of TZM during 6 months before the patient’s death. Another 2 published case reports of sustained cholestasis following TZM treatment were identified; however, the sustained nature of cholestasis was not emphasized in these reports. Sixteen cases of cholestatic hepatitis/cholestasis associated with TZM were identified in the FDA spontaneous reporting system between 2007 and 2010. Information on the course of the cholestasis in these cases could not be retrieved. In the literature there are other published reports of hepatotoxicity associated with TZM that have reported reversibility upon withdrawal of the drug. Thus, TZM appears to cause different types of hepatotoxicity. Particular attention should be paid to sustained cholestasis as a very serious type of TZM-associated liver toxicity.

Keywords: cholestasis, glioblastoma multiforme, hepatotoxicity, temozolomide.

Glioblastoma multiforme (GBM) is the most frequent malignant brain tumor in adults. GBM patients have a poor prognosis, with a mean survival time of about one year from the time of diagnosis.1 Radiotherapy in combination with temozolomide (TZM) is currently established as the first-line adjuvant treatment for GBM patients. TZM is an imidazotetrazine derivative and a pro-drug that is nonenzymatically converted to the alkylating agents 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) and 4-amino-5-imidazole-carboxamide.2 Efficacy of TZM in GBM patients has been shown in a previous phase III clinical trial that compared fractionated radiotherapy plus adjuvant TZM administration with fractionated radiotherapy alone.3 This study demonstrated a significant improvement in the median survival time of the patients treated with TZM. Likewise, a 5-year follow-up study showed that adjuvant radiochemotherapy and TZM provided significant improvements in progression-free and overall survival rates, respectively.3 TZM was approved in 1999 by the FDA and by the European Medicines Agency and is indicated for the treatment of adult patients with newly diagnosed GBM in...
combination with focal radiotherapy (concomitant phase) followed by up to 6 cycles of TZM monotherapy (monotherapy phase) for adult and pediatric patients 3 years of age or older with recurrent or progressive malignant glioma. The major toxicities of TZM are the typical ones of alkylating agents. Major hematological side effects (grade 3 to 4) are lymphopenia, thrombocytopenia, neutropenia, and leucopenia. Other very commonly reported adverse reactions (≥10% incidence) according to the FDA Prescribing Information (PI) include alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, abnormal coordination, viral infection, amnesia, and insomnia. Under postmarketing experience, the FDA PI mentions that cases of hepatotoxicity have been reported, including elevation of liver enzymes, hyperbilirubinemia, cholestasis, and hepatitis. The purpose of this report is to present a case of severe sustained cholestatic hepatitis following TZM treatment and to present a review of cases reported to the FDA Adverse Event Reporting System (AERS).

Methods

Patient data were collected in the Center for Pharmacovigilance–Berlin Case-Control Surveillance Study of Serious Rare Diseases with Possible Drug Etiology (PVZ-FAKOS), which is supported by the Federal Institute for Drugs and Medical Devices (BfArM) in Germany. The aim of PVZ-FAKOS (with a source population in Berlin of 2.9 million adult inhabitants) is to generate signals of rare adverse drug reactions in the postmarketing period. A detailed methodology of the project can be found elsewhere.

Our analysis of the AERS database of the FDA comprised the period between the first quarter of 2007 and the last quarter of 2010. The first search criteria were the names of TZM-containing drugs: “Temozolomide,” “Temodal,” “Temodar,” and “Temozomide.” We used the TZM drugs labeled “primary suspect.” TZM drugs specified as “secondary suspect” or “concomitant” were not considered in our analysis. The second search criteria were adverse events listed as preferred terms in the Medical Dictionary for Regulatory Activities (MedDRA). Reports containing the following terms were analyzed: “hepatic failure,” “acute hepatic failure,” “hepatitis,” “hepatitis acute,” “hepatitis fulminant,” “hepatic function abnormal,” “hepatic encephalopathy,” “hepatotoxicity,” “hepatitis cholestatic,” “cholestasis hepaticus,” “cholestasis,” “hepatic steatosis,” “hepatic enzyme increased,” “hepatic necrosis,” “cholelithiasis,” “hepatic lesion,” “liver disorder,” “liver injury,” “jaundice,” “hepatic injury,” “cholestatic liver injury,” and “jaundice cholestatic.” Multiple reports of the same adverse event were identified and consolidated by linking the Individual Safety Report (ISR) code, unique for every single report, with a case report (CASE) code, unique for every single case of adverse event.

Case Report

A 51-year-old male came to the emergency room in mid-July 2008 because of impaired vision to the left side, gait disturbance, and right-sided temporal headache. The patient reported that he had noticed the first symptoms a couple of days ago. Ophthalmologic examination revealed homonymous hemianopsia to the left side. Magnetic resonance tomography (MRT) demonstrated a right occipital lesion suspect of a malignant brain tumor. Serum level of aspartate aminotransferase (AST) was normal (29 U/L). After tumor resection, histological examination of the tumor confirmed the diagnosis of GBM. Immediately after the surgery, therapy was initiated consisting of dexamethasone (initially 2 mg 3 times a day and then 1 mg/day) for prevention of brain edema, pantoprazole (40 mg once a day) for prevention of gastroduodenal ulcer, and paracetamol (1000 mg 3 times a day) for postoperative pain. By the end of July, the patient was discharged from the hospital, and paracetamol was discontinued.

In mid-August 2008, fractionated radiotherapy (total dose 60 Gy, fractionated 5 × 2 Gy per week) with concomitant TZM (75 mg/m² per day p.o.) was initiated. The radiochemotherapy regimen was completed by the end of September 2008. The day after completion, the patient complained of nausea and vomiting. On the following day, he developed generalized jaundice. At the beginning of October 2008, laboratory investigations revealed a markedly increased serum level of alanine aminotransferase (ALT): 639 U/L (10–50 U/L); a moderately increased AST: 109 U/L (10–50 U/L); and increased alkaline phosphatase (AP): 261 U/L (<130 U/L), with an ALT/AP ratio of 1.75, indicating cholestatic hepatitis. Total bilirubin was markedly increased at 12.77 mg/dL (<1 mg/dL).

During follow-up care, serum levels of hepatic enzymes and of bilirubin were determined by the general practitioner (Fig. 1). Because of persisting jaundice, the patient was referred to a hepatologist for further diagnosis. Sonographic examination of the abdomen did not reveal pathologies of the liver or of the biliary tract.

By the end of October 2008, the patient went to the neurosurgery outpatient clinic for a postoperative follow-up. Because the liver injury was considered as possibly drug-induced, it was recommended not to proceed with the adjuvant TZM as monotherapy. One week later, the patient returned to the neurosurgery outpatient department and an MRT of the brain did not reveal a recurrence of the tumor. At the beginning of December 2008, the patient was again referred to the neurosurgery department because of progressive visual disturbances. A tumor relapse was excluded by MRT. At this time point, the physical examination revealed generalized jaundice and the ultrasound examination showed a modestly enlarged liver without signs of
biliary obstruction. The laboratory investigations showed only modestly elevated transaminases (ALT: 166 U/L, AST: 87 U/L), whereas the total bilirubin remained markedly elevated at 18.2 mg/dL. The prothrombin time was within the normal range. The patient was referred to the gastroenterology department for further diagnostics. Viral etiology of liver disease (hepatitis B, hepatitis C, cytomegalovirus, and Epstein–Barr virus) was excluded by serological testing. Tests were negative for autoimmune markers (antinuclear antibodies, antimitochondrial antibodies, liver-kidney microsomal antibodies, liver cytosol type 1 antibodies, soluble liver antigen/liver-pancreas antigen antibodies, liver membrane antibodies, and smooth muscle antibodies). Sonographic and MRT examination of the abdomen did not reveal any pathological findings, in particular no signs of liver or biliary tract abnormalities. The finding of a partial pancreas divisum was considered unrelated to the patient’s liver disease. Liver biopsy was performed and histological examination showed a pattern of cholestatic hepatitis (Fig. 2). Morphologic changes were described as canalicular cholestasis, feathery degeneration, and necrosis of some hepatocytes, as well as faint portal infiltration with lymphocytes, plasma cells, and some polymorphonuclear leukocytes. Bile ductules were intact, and there was no evidence for intrahepatic or extrahepatic bile duct obstruction. In the absence of histological and clinical evidence for a viral, autoimmune, alcoholic, or other toxic etiology, the findings were considered consistent with toxic liver injury most likely caused by TZM.

A therapy regimen including silymarin, ursodeoxycholic acid, and prednisolone was initiated but failed to show any therapeutic benefit. During hospitalization, the bilirubin values remained elevated within the range of 18.2 mg/dL to 23.6 mg/dL. By the end of January 2009 neurological symptoms (vertigo, gait disturbances, and lateropulsion) worsened. By the end of February 2009, the patient was referred to the Neurology Department of the University Hospital, where a tumor relapse was diagnosed. The patient was again referred to the Department of Radiotherapy. Laboratory findings from April 2009 showed bilirubin of 14.3 mg/dL (conjugated bilirubin 11.2 mg/dL), ALT: 286 U/L, AST: 75 U/L, and AP: 545 U/L. A few days after hospital discharge in April 2009, the patient died.

Fig. 1. Time course of serum levels of ALT (upper graph), AST (middle graph), and total bilirubin (lower graph).

Fig. 2. Liver parenchyma with intracellular and canalicular cholestasis associated with feathery degeneration of hepatocytes (A and B) and foci of lobular inflammation (B). Paraffin sections, hematoxylin & cosin stain; original magnification ×20.
was confirmed by histopathological examination of a strongly suggested cholestatic hepatitis. This diagnosis was supported by the results of laboratory tests that revealed significantly elevated serum levels of liver enzymes and bilirubin. The pattern of liver enzyme levels (ALT, AST) strongly suggested cholestatic hepatitis. This diagnosis was confirmed by histopathological examination of a liver biopsy that did not suggest an autoimmune, viral, or biliary etiology.

We consider that the liver injury in this case is probably causally related to TZM exposure. First of all, the manifestation of liver disease was in a close temporal relation to preceding drug treatment. Second, previous liver disease was excluded as well as other disease associated with disturbed liver function, alcohol or illicit drug abuse, and previous consumption of prescription-free synthetic drugs or herbal medicines as possible alternative causes of current liver disease. Third, serological testing for viral etiology of liver disease was negative. Fourth, serological testing for an autoimmune disorder possibly underlying the hepatitis was also negative. Fifth, repeated ultrasonography and MRT investigations did not indicate any other alternative explanation of liver injury, such as mechanical bile duct obstruction. Furthermore, there was no evidence of hemolysis that could explain the increased bilirubin serum levels. Before developing hepatitis, the patient received TZM (75 mg/m² per day) as a component of the radiochemotherapy regime. Additionally, he received a low dose of dexamethasone (1 mg/d) and pantoprazole (20 mg/d). Incidentally, this drug treatment was completed the day before the onset of clinical symptoms. The cholestatic hepatitis had a sustained course over more than 6 months and the liver pathology was clearly consistent with toxic liver injury, confirming the possibility of drug etiology. The temporal pattern of serum parameters was interpreted as initial hepatocellular damage, which was followed by sustained cholestatic liver disease.

The Drug Commission of the German Medical Association published another case of TZM-associated cholestatic hepatitis and sustained hepatotoxicity that was reported to its spontaneous reporting system.7 This case concerned a 67-year-old male with a right-central GBM. After surgery and the start of a radiochemistry regime with TZM, the patient developed jaundice, a 3-fold elevation of AST, a 10-fold elevation of ALT, a 9-fold elevation of gamma glutamyl transferase, and a bilirubin level of 11.1 mg/dL. TZM was discontinued, as the possible cause of this liver injury. Despite TZM discontinuation, the level of bilirubin continued to increase up to 24 mg/dL. The patient developed hepatic failure with encephalopathy (Quick value 52%, International Normalized Ratio 1.6) and eventually died.

A search for further cases of hepatotoxicity associated with TZM in the PubMed and Embase databases (as of September 6, 2011) identified 7 cases,8–14 of which 2 were considered not causally related to TZM but to other drug treatment (valproic acid, rifampin) given concomitantly with TZM.9,13 Among the 5 cases thought to be causally related to TZM, one published by Goldbecker and colleagues10 concerned a 66-year-old woman who developed severe cholestatic liver damage after 27 cycles of radiation combined with continuous TZM treatment for GBM. At the start of radiochemistry in August 2007, liver enzymes were in the normal range. In October 2007 the patient developed liver injury after 27 cycles of radiation combined with continuous TZM treatment for GBM. At the start of radiochemistry

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<th>MedDRA-preferred term</th>
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<td>Hepatic encephalopathy</td>
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### FDA Review

Our analysis of the FDA AERS database for the years 2007–2010 retrieved 198 single adverse event reports of TZM-associated hepatotoxicity corresponding to 154 single cases. In these 154 unique cases, 110 had a single hepatic adverse event reported and 44 had more than one. Our above-described case was also included in the FDA AERS database. The MedDRA-preferred terms of hepatic adverse events reported in association with TZM as the primary suspect drug are described in Table 1. The most frequent adverse hepatic event related to TZM was “hepatic function abnormal,” with 48 single reports.

### Discussion

Here, we describe the case of a severe sustained cholestatic liver injury that occurred in close temporal relation to TZM treatment in a GBM patient. Importantly, this liver injury was not associated with a previous or concurrent liver disease. The patient did not have a previous history of drug allergy or elevated bilirubin values in the past. The onset of clinical symptoms coincided with the end of the administration of TZM. Early clinical symptoms presented by the patient were consistent with acute liver disease. Hepatic injury was confirmed by the results of laboratory tests that revealed significantly elevated serum levels of liver enzymes and bilirubin. The pattern of liver enzyme levels (ALT/ AP ratio) strongly suggested cholestatic hepatitis. This diagnosis was confirmed by histopathological examination of a liver biopsy that did not suggest an autoimmune, viral, or biliary etiology.

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jaundice, cholestatic enzymes were massively elevated, and a liver biopsy showed cholestasis. The etiology was suspected to be drug-toxic, most probably due to TZN. During the course of her disease, the patient developed hepatic encephalopathy before she died of intracerebral bleeding.10 Another 2 cases were reported as activation of viral hepatitis B during TZN use, one of them with fatal outcome in a patient who was concomitantly taking valproic acid, which, according to the FDA, interacts with TZN to reduce its clearance,8,11 and the last 2 cases were reported as drug-induced hepatitis, one under TZN plus indomethacin and the other under TZN plus lomustine;12,14 both cases required treatment discontinuation.

In our case as well as in the published case with severe cholestatic hepatitis and hepatic encephalopathy,10 TZN was coadministered with pantoprazole. Even though pantoprazole is known to induce elevation of liver enzymes, according to a recent review by Thomson et al.,15 pantoprazole has not been associated with hepatitis. Furthermore, before TZN was initiated, our case had received pantoprazole for 4 weeks without signs of liver toxicity.

The underlying mechanism of this rare, serious liver toxicity remains unclear. Discussed mechanisms of drug-induced liver injury include apoptosis activated by tumor necrosis factor–α, inhibition of mitochondrial function, and neoantigen formation.16 Apart from age and sex, genetic polymorphisms of drug-metabolizing enzymes such as cytochrome P450 have been discussed as risk factors for drug-induced liver injury.16 According to the FDA PI,5 cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and its active metabolite MTIC, since TZN and its active metabolite are spontaneously hydrolyzed at physiologic pH values.

Our case appears to be the third published case of severe sustained cholestatic hepatitis associated with TZN, although the 2 other case reports7,10 have not emphasized the aspect of sustained cholestatic liver damage. Considering the other published case reports, TZN appears to cause different types of hepatotoxicity with regard to reversibility. Sustained cholestasis after withdrawal of TZN is alarming and should receive close attention and better reporting. From our review of the FDA AERS database, we identified 16 unique cases of cholestatic hepatitis or cholestasis associated with TZN. Unfortunately these reports do not provide information about whether the liver damage was sustained or not.

### Conclusion

TZN appears to be associated with different types of liver toxicity. Sustained cholestatic hepatitis is a rare but very serious liver toxicity associated with TZN that merits close attention. Careful monitoring of clinical and laboratory signs of hepatic injury is warranted in patients receiving TZN. Further reports of cases of TZN-associated liver toxicity are needed to better characterize the spectrum of TZN-associated hepatotoxicity and its impact on the benefit/risk profile of this drug.

### Acknowledgments

The case presented here was identified within the PVZ-FAKOS study. We thank hospitals, clinicians, and collaborators who contributed to data collection.

### Conflict of interest statement

None declared.

### Funding

The PVZ-FAKOS study is supported by the Federal Institute for Drugs and Medical Devices (BfArM).

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