Mycophenolate Mofetil Hepatotoxicity Associated With Mitochondrial Abnormality in Liver Transplant Recipients and Mice

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ABSTRACT

Objectives: Mycophenolate mofetil (MMF) is a widely used immunosuppressive agent. MMF hepatotoxicity has been reported in non-transplant and renal transplant patients with minimal histologic description. This is the first study describing detailed histology and ultrastructure of MMF hepatotoxicity.

Methods: Four liver-transplant recipients (Cases 1–4) were suspected to have MMF hepatotoxicity. Cases 1–3 (two females and one male; 4–17 years) had multiple biopsies for liver function test (LFT) abnormalities. Case 4 (female; 16 years) had a surveillance biopsy. Electron-microscopic examination (EM) was requested on Cases 1–3 for unexplained, persistent LFT elevation and histologic abnormalities despite therapy and Case 4 for unexplained histologic abnormalities despite a stable clinical course. To confirm the pathologic changes in the human allografts, livers from MMF-treated and untreated mice were also reviewed.

Results: While the allograft biopsies showed nonspecific histologic changes, EM revealed unequivocal mitochondrial abnormalities similar to those seen in primary and secondary mitochondrial disorders. In Cases 1 and 2, LFTs improved after stopping and reducing MMF, respectively. In Case 3, pre- and post-MMF treatment biopsies were performed and only the post-MMF biopsy demonstrated mitochondrial abnormalities. Mitochondrial abnormality in Case 4 was subclinical. The mouse study confirmed that MMF caused various stress changes in the mitochondria; number of mitochondria/cell (mean ± standard deviation; untreated group: 58.25 ± 8.426; MMF-treated group: 76.37 ± 18.66), number of lipid droplets/cell (untreated: 0.9691 ± 1.150; MMF-treated: 3.649 ± 4.143) and sizes of mitochondria (μm, untreated: 0.8550 ± 0.3409; MMF-treated: 0.9598 ± 0.5312) were significantly increased in hepatocytes in the MMF-treated mice compared with the untreated mice ($P < 0.0001$).

Conclusions: Although MMF is safe for the majority of patients, MMF can cause mitochondrial stress, which may trigger more severe mitochondrial abnormalities in a small subset. MMF hepatotoxicity should be considered for MMF-treated patients with unexplained, persistent LFT abnormalities and nonspecific histologic findings. EM should be requested for these cases.

Key Words: drug-induced liver injury, histopathology, liver transplant, mitochondria, mycophenolate mofetil, ultrastructure (electron microscopy)

An infographic is available for this article at: *http://links.lww.com/MPG/C361*.

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What Is Known

- Recognition of drug-induced liver injury (DILI) can be challenging because DILI displays diverse, often nonspecific laboratory and histopathologic changes.
- Rare cases with mycophenolate mofetil (MMF) hepatotoxicity have been reported in non-transplant and renal transplant patients.

What Is New

- This is the first study reporting detailed histopathology and ultrastructure of MMF hepatotoxicity.
- Despite nonspecific histologic abnormalities, electron-microscopic examination (EM) revealed unequivocal mitochondrial abnormalities similar to those seen in primary and secondary mitochondrial disorders.
- MMF hepatotoxicity should be considered for MMF-treated patients with unexplained, persistent liver enzyme abnormalities and nonspecific histology.
- EM should be requested for these cases.

Drug-induced liver injury (DILI) represents the leading cause of acute liver failure in the United States (1–3) with an estimated incidence of 1 in 10,000 and 1 to 100,000 patients (4). Recognition of DILI can be challenging clinically (3) and histologically (5) because DILI can be present with highly diverse laboratory and histologic changes. The histologic patterns of DILI include hepatitis, cholestasis, granulomatous inflammation, macro- and/or micro-vesicular steatosis with or without steatohepatitis, hepatocellular necrosis ranging from single cell drop-out to broad necrosis, sinusoidal obstruction/veno-occlusive disease, and any combination of these injury patterns. Additionally, DILI frequently displays nonspecific (unclassifiable) pathologic changes (5). These diverse,

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often nonspecific histologic patterns make it difficult to establish practical diagnostic criteria for DILI.

To treat or prevent acute graft rejection in solid organ recipients, azathioprine, 6-mercaptopurine (6-MP), cyclophosphamide, and calcineurin inhibitors, such as cyclosporine and tacrolimus, have been used in combination with high-dose corticosteroids. Mycophenolate mofetil (MMF) and sirolimus have emerged recently as additional immunosuppressive agents in managing solid organ transplants (6,7). These immunosuppressive agents are often used in combination with other medications and they may be also used to treat patients with pre-existing liver disease, such as autoimmune hepatitis. DILI can be associated with any of these immunosuppressive agents. Among these agents, azathioprine- and 6-MP-related hepatotoxicity are well-known and account for 1–2% of DILI. They typically show cholestatic, hepatocellular and mixed injury patterns (8). Otherwise, the injury is thought to be generally mild and only a small number of cases have been reported (9). The number of cases may be undercounted because it is often difficult to diagnose DILI associated with immunosuppressive agents and determining a particular causative agent is further challenging.

MMF is an immunosuppressive agent commonly used as an adjunctive agent to prevent and/or treat acute cellular rejection (ACR) in solid organ transplant recipients and as a therapeutic agent in non-transplanted patients with various diseases with immune dysregulation. Major adverse effects of MMF include bone marrow suppression, gastrointestinal, neurological symptoms and teratogenicity. Rare sporadic cases with MMF-related hepatotoxicity have been reported in the native livers of non-transplant patients and renal transplant recipients; however, these reports described no or only minimal histopathology and ultrastructural analysis had not been performed (10–16).

Children's Hospital Los Angeles (CHLA) performs 110–120 transplant liver biopsies per year. Liver biopsy is routinely performed on transplant recipients with elevated liver function tests (LFTs) and as a surveillance biopsy for patients with normal LFTs in accordance with internal protocols. The predominant indication for allograft biopsy is to rule out ACR as it is the most frequent clinical concern and requires prompt treatment. ACR is diagnosed according to the standardized histologic criteria defined by the Banff Working Group (Banff criteria) (17); however, other possible etiologies of liver dysfunction, which are often evident only as nonspecific histologic findings on liver biopsies, are common yet often receive little consideration in pathology reports.

We herein present four liver transplant patients treated with MMF, who were clinically suspected to have MMF hepatotoxicity. This is the first study that shows the detailed histopathology and ultrastructure of MMF hepatotoxicity in human liver allografts and mouse livers treated with MMF.

METHODS

Patients' Transplant Liver Biopsies

The study has been approved by our internal Institutional Review Board (IRB) at CHLA. Liver biopsies were performed by interventional radiology at CHLA. Biopsies were immediately fixed in 10% formalin (Medical Chemical Cooperation, Torrance, CA, USA) for light-microscopic examination (LM) and 2.5% buffered glutaraldehyde (BCC Biochemical, Mount Vernon, WA, USA) for electron-microscopic examination (EM).

All staining and histologic examination were performed at the Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory at CHLA. For LM, we performed hematoxylin & eosin (H&E) and special staining (Periodic acid-Schiff [PAS], PAS with diastasis [PASD], reticulin, iron and trichrome) per biopsy according to our operating procedure. Ultrastructural analysis was performed as previously described (18,19).

ACR was diagnosed and scored using the rejection activity index (RAI), which grades: portal inflammation (score 1–3), bile duct damage (score 1–3), and venous endothelial inflammation (score 1–3). Each score was added and the degree of ACR was scored as follows: RAI = 0–9; <3: borderline/indeterminate ACR, 3–4: mild ACR, 5–7: moderate ACR and >7: severe ACR (17).

Nonspecific (unclassifiable) hepatocellular injury is often referred to ''reactive changes''. Histologic features of ''reactive changes'' include a combination of enlarged hepatocytes with hydropic changes (expanded, pale to clear cytoplasm) and coarse eosinophilic granules (eg, mega-mitochondria), nuclei with anisonucleosis and bi-/multi-nucleated forms, cholestasis, steatosis and/or necrosis. Necrosis can range from single cell necrosis (acidophil bodies) to rarely broad necrosis with collapsed lobules.

Mouse Liver Samples

The study has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Cincinnati and CHLA. All of the animal experiments in the present study were compliant with relevant ethical regulations regarding animal research. Mice were 7–8 months old female, which were housebred from C57BL/6 mice (the Jackson Laboratory, Bar Harbor, ME, USA). For the MMF-treated group, mice (7–8 months of age) were given MMF by oral gavage. MMF was dissolved in 0.5% methylcellulose/0.1% Tween 80 solution and 120mg/kg/day was administrated orally. For untreated group, 0.5% methylcellulose/0.1% Tween 80 solution was used for vehicle treatment. Two of the five MMF-treated mice died on days 12 and 13 of the treatment and were excluded from the experiment. The rest were sacrificed on day 14 and necropsy was performed. Livers were harvested and immediately fixed for LM and EM as described above.

H&E and trichrome staining were performed on each liver section from the three MMF-treated mice and the five untreated mice at CHLA. Ultrastructural analysis was performed on the mouse liver tissue from the three MMF-treated mice and three mice randomly selected from the untreated group, according to the method described above. EM images were captured digitally at the same magnification (8000×). Thirty hepatocytes per mouse liver were randomly selected and numbers of the mitochondria and lipid droplets were counted per hepatocyte using digital images. The greatest dimensions of randomly selected 50 mitochondria per hepatocyte from each mouse were measured using image analysis software, Cellsens (Olympus, Tokyo, Japan).

Data Analysis (Mouse Livers)

The number of mitochondria and lipid droplets per hepatocyte (20 cells per mouse, total 60 cells per group) and size of the hepatocellular mitochondria (50 mitochondria per hepatocyte; total 60 cells/3000 mitochondria per group) from the MMF treated and untreated mice were compared by repeated measures mixed model analysis, with mice as a random effect and group as fixed, at a 0.005 significance level, using Prism8 software (GraphPad Software, San Diego, CA, USA).

RESULTS

Case Reports

The study included three female and one male liver transplant recipients. Clinical demographics of the patients are summarized in Table 1. Three MMF-treated liver transplant recipients

TABLE 1. Clinical demographics and biopsy results of Cases 1–4

Case no.	Age	Sex	Transplant	Biopsy: Time after Tx	Biopsy: Reason for Tx	Biopsy: Biopsy no.	Biopsy: Timing of biopsy from the initial biopsy of the event	EM: EM performed	EM: EM features	Pathology report: RAI reported: Total	Portal	Duct	Endothelial	Other findings reported: Fibrosis	Lobular inflammation	Additional features	Additional testing
1	12 y	F	Cadaveric whole liver	2 mo	BA, end-stage liver disease, portal hypertension	1	Day 1	No	N/A	3	1	1	1	None	Rare mild	N/A	C4d: neg
						2	Day 8	No	N/A	2	1	1	0	None	Focal neutrophilic infiltrates	N/A	Viral study and one HSV, EBER.): CMV, EBER, HSV1/2: neg): neg
						3	Day 20	Yes	Mitochondrial pleomorphism, Crystalloid inclusions	2	1	1	0	Focal mild perisinusoidal and periportal	Minimal scattered	Diffuse reactive changes, mild sinusoidal dilatation	C4d: neg
2	4 y	F	Cadaveric whole liver	2 mo	Hepatoblastoma	1	Day 1	No	N/A	5	2	1	2	none	Occasional mild	N/A	CMV, adenovirus, HSV1/2 EBER: neg
						2	Day 12	No	N/A	3	1	1	1	none	Rare mild	N/A	N/A
						3	Day 25	No	N/A	3	1	1	1	Mild periportal (stage 1)	Scattered single cell necrosis	N/A	CMV, adenovirus, HSV1/2 EBER: neg
						4	Day 42	Yes	Mitochondrial pleomorphism Crystalloid inclusions	3	1	1	1	None	None	Reactive changes with lipofuscin, mild sinusoidal dilatation	CMV EBER, C4d: neg
3	17y	M	Cadaveric whole liver	3mo	CDG	1	Day 1	No	N/A	3	1	1	1	Mild portal fibrosis (stage 1)	Rare mild	N/A	CMV, adenovirus, HSV1/2, EBER, C4d: neg
						2	Day 6	Yes	Normal	3–4	1–2	1	1	Perisinusoidal fibrosis (stage 0)	None	Mild zone 3 dilatation	C4d: neg
						3	Day 21	Yes	Mitochondrial pleomorphism, Crystalloid inclusions	3	1	1	1	Perisinusoidal fibrosis (stage 0–1)	None	Diffuse reactive changes	C4d: neg
						4	Day 37	No	N/A	3	1	1	1	None	None	Reactive changes and scattered acidophil bodies	CMV, adenovirus, HSV1/2, EBER, C4d: neg
4	16y	F	Cadaveric split (left lateral segment)	13y	BA, end-stage liver disease	1	Day 1	No	N/A	2	1	0	1	Portal, perisinusoidal with focal bridging (Stage 2–3)	None	N/A	CMV, adenovirus, HSV1/2, EBER: neg
						2	Day 342	No	N/A	3	1	1	1	Portal, perisinusoidal with focal bridging (Stage 2–3)	Focal, mild	Focal hepatocyte dropout	None
						3	Day 509	Yes	Mitochondrial pleomorphism, Crystalloid inclusions	2	1	0	1	Portal, perisinusoidal with focal bridging (Stage 2–3)	None	Microvesicular steatosis (30%)	EBER: neg

Note that ''additional features'' in the pathology reports were not standardized among pathologists and did not affect the final diagnoses. BA = biliary atresia; CDG = congenital disorder of glycosylation; CMV= cytomegalovirus; Duct = bile duct damage score; EBER = Epstein-Barr encoding region in situ hybridisation; Endothelial = venous endothelial inflammation score; HSV = herpes simplex virus; Kasai = Kasai procedure; mo = months; N/A = not applicable; neg = negative; Portal = Portal inflammation score; RAI = rejection activity index; Tx = transplant; y = years.