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Rapid Report

Tolvaptan- and Tolvaptan-Metabolite-Responsive T Cells in Patients with Drug-Induced Liver Injury

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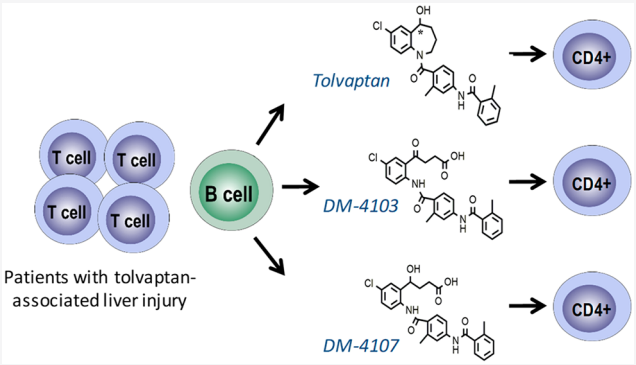
ABSTRACT: Tolvaptan is an effective drug for the treatment of autosomal dominant polycystic kidney disease, but its use is associated with a significant risk of liver injury in a small number of patients. Herein we describe the presence of tolvaptan- and tolvaptan-metabolite-responsive T cell clones within the peripheral circulation of patients with liver injury. Drug treatment of the clones resulted in a proliferative response and secretion of IFN-γ, IL-13, and the cytolytic molecule granzyme B. Future work should explore pathways of tolvaptan driven T cell activation and the role of T cells in the disease pathogenesis.



Tolvaptan-associated liver injury was first detected in two prospective, randomized, phase 3 clinical trials investigating its use in the treatment of autosomal dominant polycystic kidney disease: Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes (TEMPO) 3:4 (NCT00428948) and the open-label follow-on trial TEMPO 4:4 (NCT01214421).[1] An independent, blinded, expert Hepatic Adjudication Committee (HAC) reviewed characteristics of positively adjudicated cases and proposed a potential signature pattern of the observed events. A delayed onset of liver injury was observed between 3 and 18 months after the start of tolvaptan treatment, along with rapid recurrence of symptoms following rechallenge, which is a hallmark feature of an adaptive immune mediated pathogenesis.[1] Corroborating this notion are data generated from a genetically diverse mouse population and a human hepatocyte study that indicated immunological perturbation by tolvaptan,[2,3] leading to speculation about the role of the adaptive immune response in the etiology of tolvaptan-associated liver injury. Drug-responsive T cells have recently been identified in blood of patients with other forms of drug-induced liver injury (e.g., flucloxacillin, amoxicillin clavulanate),[4,5] and in the case of flucloxacillin, T cell infiltrates were identified in a liver biopsy.[6] Thus, in this study we utilized peripheral blood mononuclear cells (PBMCs) from patients with tolvaptan-associated liver injury to explore whether drug-responsive T cells are detectable and, if so, to define the nature of the chemical entity that stimulates the immune cells.

Blood was collected from nine subjects with autosomal dominant polycystic kidney disease who experienced liver injury in the TEMPO 3:4 or TEMPO 4:4 trial. Subjects were retrospectively enrolled in a specimen collection clinical trial. Informed written consent was obtained from the study subjects. Lymphocyte transformation tests were performed as previously described with tolvaptan (1−60 μM) and two metabolites (structures shown in Figure 1), an oxybutyric acid derivative (DM-4103; 5−90 μM) and a hydroxybutyric acid derivative (DM-4107; 10−500 μM), with tetanus toxoid as a positive control.[7] Drug concentrations were selected by inhibition of mitogen-induced proliferation. Bulk cultures were generated through 14 day culture of PBMCs (2×10^6 cells/well; 1 mL) in the presence of each compound. On days 6 and 9, cultures were supplemented with 200 IU/mL of IL-2. T cell clones (TCCs) were isolated via limiting dilution and expanded through mitogen stimulation.[7] TCC specificity was assessed by culturing 5×10^4 TCCs and 1×10^4 autologous irradiated EBV-transformed B-cells (antigen-presenting cells (APCs)) per well incubated with the relevant compound for 48 h. [^{3}H]thymidine (0.5 μCi/well) was added for 16 h, and compound-specific proliferation was assessed. TCCs with stimulation indices (SIs) (proliferation in the presence of compound/proliferation in control wells) above 1.5 at this stage were deemed compound-responsive and were subjected

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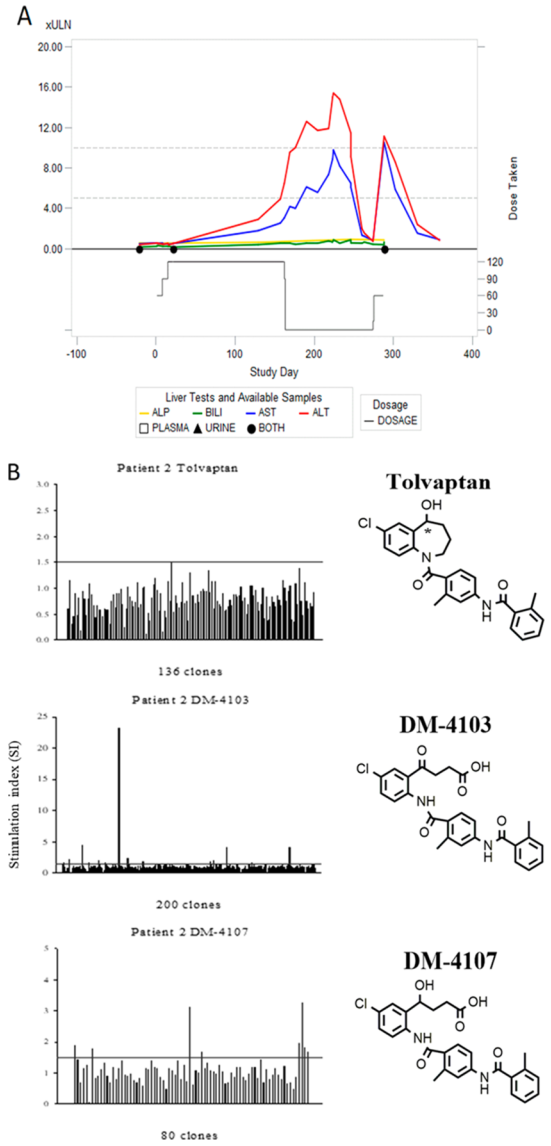

Figure 1. Elevated hepatic enzymes from trial subjects and specificity testing of TCC. (A) Liver function over the study period for a trial subject that was rechallenged at a lower dose. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (BILI) are indicated by traces in the upper compartment. The dose regimen is depicted in the lower compartment. (B) TCC specificity screens of one trial subject (three are shown in Supplementary Figure 1). TCCs (0.5×10^5) were cultured with tolvaptan, DM-4103, or DM-4107 and EBV-transformed B-cells (APCs) (0.1×10^5) for 48 h at 37 °C, 5% CO_2. [^{3}H]thymidine (0.5 μCi/well) was added for the last 16 h of incubation before plates were harvested and proliferation was measured.

to cross-reactivity assessment and analysis of cytokine secretion using ELIspot.

Causality assessment of tolvaptan in trial subjects was determined through the expert opinion of the HAC (the gold standard for causality assessment of drug-induced liver injury). For all trial subjects recruited to this study, the role of tolvaptan in the manifestation of liver injury was adjudicated as at least "probable" (the preponderance of evidence supports a link between the drug and the liver injury) by the HAC (Table 1). All patients exhibited a positive dechallenge, that is, the liver injury was resolved with the cessation of tolvaptan

Table 1. Trial Subject Demographics and Details of the Adverse Event[a]

patient	region	sex	age at max ALT	max ALT (xULN)	category	dose at max ALT (TTD mg)	simul. Cmax (ng/mL)	simul. AUC (h·ng/mL)	TKV at baseline	TKV at max ALT	eGFR at baseline	eGFR at max ALT	liver cysts ?[b]	dechallenge	rechallenge	adjudication	time to LTT after ADR	LTT result	cloning
1	Europe	M	40	11.8	ALT > 7x	60	389	5956	1170	1177	108.2	73.7	yes	+ve	N/A	probable	0	−ve	+ve
2	Asia	F	51	11.1	ALT > 7x	120	586	7666	607	631	57.0	68.6	yes	+ve	+ve	probable	0	−ve	+ve
3	Asia	M	43	9.47	ALT > 7x	60	nd	nd	1192	1231			yes	+ve	N/A	probable	0	−ve	+ve
4	Asia	F	46	25.9	Hy's law	120	540	5396	697	766	52.3	57.1	yes	+ve	N/A	probable	0	−ve	+ve
5	Europe	F	30	10.4	ALT > 7x	120	458	4417	1017	1011	108.1	100.8	unk	+ve	N/A	highly likely	0	−ve	+ve
6	Asia	M	45	38.8	ALT > 7x (elev Bil)	60	402	5043	452	444	89.9	90.2	yes	+ve	N/A	probable	0	−ve	+ve
7	Asia	F	41	22	ALT > 7x	60	229	2488	708	630	81.3	71.9	yes	+ve	N/A	probable	0	−ve	−ve
8	Americas	F	35	14.7	Hy's law	120	657	9530	615	nd[b]	99.5	83.7	unk	+ve	N/A	probable	0	−ve	N/A
9	Americas	F	33	7.7	ALT > 7x	120	487	5643	1336	1818	35.7	37.7	unk	+ve	+ve	probable	0	−ve	−ve

[a]nd = not done; unk = unknown (no diagnosis of liver cysts at baseline assessment). [b]Discontinued before next assessment.

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treatment. A recurrence of injury was observed in the two subjects rechallenged with tolvaptan, with a more rapid onset. Monitoring of drug-induced liver injury was conducted through the temporal use of liver function tests, depicted as time-course plots superimposed over the dosage regimen. A plot from one of the rechallenged patients is depicted in Figure 1A. Although five subjects of Asian origin and four of non-Asian origin were included in our study, to date there has been no evidence of susceptibility to tolvaptan-related liver injury based on ethnicity or race.

PBMCs from the trial patients were not stimulated to proliferate with tolvaptan, DM-4103, or DM-4107. Proliferative responses were detected with tetanus toxoid, confirming the viability of PBMC recall responses. The lymphocyte transformation test is known to possesses limited application with certain cutaneous manifestations such as SJS/TEN despite the fact that drug-responsive T cells are readily detectable in inflamed skin.[8] Likewise, inconsistent results have been reported in PBMCs from flucloxacillin liver injury patients, an occurrence previously attributed to a low circulating precursor frequency of flucloxacillin-specific T cells.[4] Thus, the circulating precursor frequency need not (and often does not) correlate with the severity of the iatrogenic disease. Upon limiting dilution and cloning from PBMC bulk cultures, tolvaptan-, DM-4103-, and DM-4107-responsive TCCs were detectable in (6/8) trial subjects and were predominantly of the CD4+ phenotype. Figure 1B shows initial testing data using TCCs from one patient. Dose-dependent proliferation and IFN-γ, IL-13, and granzyme B release from a DM-4107-responsive TCC is shown in Figure 2. Low cross-reactivity with tolvaptan and DM-4103 was observed. Unfortunately, despite repeated attempts, long-term culture and expansion of TCCs from the subjects of the TEMPO trials was not possible, and therefore, further characterization was not feasible.

The data from our study as well as the clinical pattern of liver injury in subjects with autosomal dominant polycystic kidney disease from the TEMPO clinical trials provide supportive evidence for the role of the adaptive immune system in tolvaptan-associated liver injury. Secretion of IFN-γ, IL-22, and granzyme B is a common feature of T cells from patients with other forms of drug-induced liver injury.[4,5] Thus, additional studies should explore why IL-22 secretion was not detected from the DM-4107 clones. Activation of the clones was observed at greater than 10-fold higher concentrations than patient plasma concentrations. The reason for this is not known but may be that in vitro culture conditions do not accurately mimic physiological conditions. Alternatively, hepatocellular accumulation of drugs, metabolites, or protein adducts may be a critical factor in the determination of T cell activation in patients.

The pathogenesis of liver injury associated with tolvaptan (and drugs that have a similar pattern of injury) is regarded as multifaceted, with convergence of multiple risk factors cumulatively increasing the chance that a given compound will precipitate such an adverse event in an individual. Thus, while adaptive immune attack upon the liver is putatively the ultimate cause of liver injury associated with tolvaptan, the sequence of events leading up to an immune response may well be multifactorial and/or be exacerbated by autosomal dominant polycystic kidney disease etiology. The principal mechanisms proposed to date for tolvaptan and its major metabolites have been hepatocyte bile acid transporter

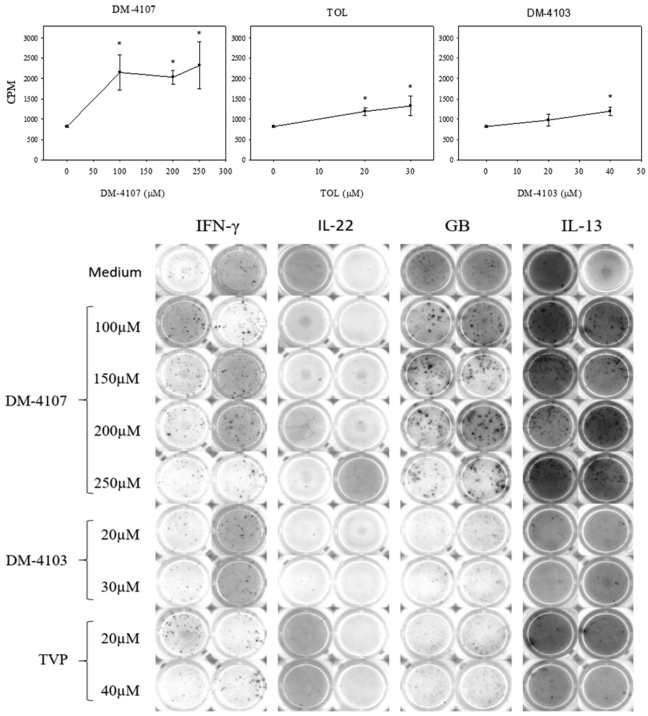

Figure 2. DM-4107-responsive TCCs proliferate and secrete cytokines in a dose-dependent manner and display low cross-reactivity with tolvaptan and DM-4103. TCCs (0.5×10^5) were cultured with tolvaptan, DM-4103, or DM-4107 and autologous EBV-transformed B-cells (0.1×10^5) for 48 h at 37 °C, 5% CO_2. [^{3}H]thymidine (0.5 μCi/well) was added for the last 16 h of incubation before plates were harvested and proliferation was measured. *, $P < 0.05$; Mann–Whitney test. Dose–response and cross-reactivity series were conducted across a cytokine panel for the same TCCs and analyzed using ELIspot.

inhibition, mitochondrial dysfunction, and oxidative stress.[2,3] It has been proposed that these early events may be more widespread but lead to overt injury only in individuals with underlying susceptibility to direct toxicity (e.g., impaired mitochondrial function) and/or harboring genetic risk factors (e.g., specific HLA alleles). In ongoing studies we are utilizing PBMCs from HLA-typed tolvaptan naive healthy donors to investigate the chemical entity that primes naive T cells and to determine pathways of drug-specific T cell activation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00328.

Supplementary Figure 1 containing initial results of T cell cloning from three trial subjects to tolvaptan, DM-4103, and DM-4107 (PDF)

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[‖]A.G. and S.H. contributed equally. S.H., A.G., and K.J. conducted the experiments. The manuscript was written through contributions of all authors. All of the authors approved the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TEMPO, Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes; HAC, Hepatic Adjudication Committee; TCC, T cell clone; PBMC, peripheral blood mononuclear cell.

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