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Activation of tolvaptan-responsive T-cell clones with the structurally-related mozavaptan

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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 T-cell-mediated liver injury in a small number of patients. An important clinical conundrum following the contraindication of tolvaptan is whether administration of agents of similar pharmacological action and structure will be tolerated. Herein, we addressed this question through the exposure of tolvaptan-responsive T-cell clones to similar pharmaceutical agents. Whilst lixivaptan and conivaptan did not activate tolvaptan-responsive T-cells, mozavaptan evoked proliferative responses comparable with tolvaptan itself, indicating that there may be collateral immunological intolerance to this compound as a product of sensitization to tolvaptan.

1. Introduction

Tolvaptan is a selective arginine vasopressin type 2 receptor antagonist that is the first-to-market to delay the progression of autosomal dominant polycystic kidney disease (ADPKD) in those patients with predicted fast progression of disease and CKD stages 1–3. However, tolvaptan elicits drug induced liver injury in a small number of patients with ADPKD (Torres, 2012; Torres, 2017). Clinical data implicates the involvement of an immunologically-mediated mechanism in tolvaptan-associated liver injury (Watkins, 2015), which was demonstrated by the recent evidence that T-cells from patients with liver injury are stimulated to proliferate with tolvaptan and two of its metabolites (DM-4103 and DM-4107) (Gibson, 2020). Furthermore, investigation of the intrinsic immunogenicity of tolvaptan and its metabolites facilitated demonstration of the compounds capacity to elicit *de-novo* responses within drug-inexperienced healthy donors (Hammond, 2021). To prime naïve T-cells, tolvaptan and its metabolites were cultured for 12 days with autologous dendritic cells. At this point the drug-enriched T-cells were isolated *via* limiting dilution and subject to repetitive mitogen-driven expansion to generate sufficient numbers of cells for assessment of cellular phenotype and function and pathways of major histocompatibility complex (MHC)-restricted drug presentation as described previously (Hammond, 2021). Given that re-exposure of individuals to a compound they are hypersensitive to can lead to further adverse reactions (Watkins, 2015), an important question arises: once an individual permanently discontinues tolvaptan, are there secondary therapeutic options in order to maintain optimal autosomal dominant polycystic disease control?

Treatments for ADPKD are under investigation that target alternate pathways other than vasopressin, such as activation of Nuclear factor erythroid2-derivative – 2 (Nrf2) (Capuano, 2021; Pergola, 2018; Testa and Magistroni, 2020) or with similar mechanism of action and molecular structure, such as other vaptan-class compounds The exploration of the potential effect of hypersensitivity to tolvaptan on the immunological tolerability of compounds of similar structural composition is important for defining safe future treatment paradigms. Of note, lixivaptan was investigated clinically in subjects previously treated with tolvaptan in a recent trial (NCT04152837). Immunological cross reactivity between compounds has been reported for a number of drugs of the same class (Handoko, 2008; Schmid, 2006; Romano, 2005). These include the anti-epileptic drugs, quinolone and β-lactam antibiotics. Thus, herein, we report utility of *in-vitro* cell culture platforms to study

Abbreviations: EBV, Epstein-barr virus.

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cross reactivity of T-cell clones identified as tolvaptan-responsive within our previous work (Hammond, 2021). Alternate vaptans studied include lixivaptan, mozavaptan and conivaptan (Fig. 1), as well as numerous other benzodiazepine derivatives in early discovery (Cao, 2022).

2. Materials and methods

T-cell clones derived from two separate donors, primed to tolvaptan as described in Hammond et al. (Hammond, 2021) were utilized for this assessment. Briefly, naïve healthy donor T-cells depleted of regulatory T-cells (2×10^5) were cultured with autologous mature monocyte-derived dendritic cells (8×10^4) and tolvaptan (25 μM) and incubated for 12 days (37 ^{0}C; 5 % CO_2). T-cells were cloned from the T-cell lines *via* serial dilution and repetitive mitogen stimulation. Epstein-Barr virus (EBV)-transformed B-cell lines were generated from the donor peripheral blood mononuclear cells *via* transformation with supernatant from the EBV-producing cell line, B95.8. EBV-transformed B-cells were used thereafter as an immortalised autologous source of antigen presenting cells. T-cell clone phenotyping was performed by flow cytometry. For dose-response and cross reactivity assessment, T-cell clones (5×10^4) were incubated with irradiated EBV-transformed B-cells (1×10^4) and relevant compound tolvaptan, lixivaptan, mozavaptan (all 1–50 μM) and conivaptan (1–25 μM) in 96-well U bottomed plates for 48 h. Wells were then pulsed with tritiated thymidine (0.5 μCi/well, 5 Ci/mmol; Morovek Biochemicals, Brea, CA) for an additional 16-hour culture period, allowing measurement of compound specific T-cell clone proliferation through ensuing incorporation.

3. Results

As described in Hammond (2021) clones displayed a CD4 + (TCC 2, 3) or CD8 + (TCC 1) phenotype and proliferated in a dose-dependent manner with tolvaptan. Fig. 2 shows three clones which were derived from two separate donors within *in vitro* cultures and which display strong, medium and weak proliferative responses in the presence of tolvaptan. Significant, dose-dependent proliferative responses of the clones were also observed for mozavaptan, whilst no responses were observed with lixivaptan or conivaptan. Clones were cultured with slightly lower concentrations of conivaptan as the highest concentration of 50 μM inhibited proliferation of phytohaemagglutinin-stimulated peripheral blood mononuclear cells (results not shown).

4. Discussion

These data support immunological cross-reactivity of tolvaptan responsive T-cell clones to mozavaptan, with insensitivity observed to lixivaptan and conivaptan. Mozavaptan is the most structurally homologous compound to tolvaptan of the vaptans investigated and therefore may exhibit cross-reactivity if dosed to patients with ADPKD that previously exhibited tolvaptan induced liver injury. However, although mozavaptan showed aquaretic effects in a pck rat disease model, both cyst number and kidney weight increased with administration, (Roix and Saha, 2013) insinuating a lack of efficacious benefit. Regardless, considerations of compound sturcture in assessing risk of susceptibility to immune cross-reactivity could be useful as a drug development tool, particularly within a class of compounds. In recent news, Centessa announced the strategic decision to discontinue clinical development of lixivaptan for ADPKD (Pharmaceuticals). This is in the wake of detection of ALT/AST elevations in the ALERT study, which evaluated the safety of lixivaptan in patients who had already discontinued tolvaptan treatment due to ALT elevations considered due to tolvaptan (NCT04152837). At present, information of the prevalence, severity and clinical pattern of lixivaptan induced liver injury in this cohort are not publicly available. Previous *in-silico* models have predicted that lixivaptan would exhibit a lower frequency of ALT elevations than tolvaptan (Woodhead, 2020), which is complemented by the current TCC data. These data demonstrate cross-sensitization to compounds that are structurally similar and thus serve as a step towards identification of structural risk factors applicable to secondary therapeutics for individuals who experience tolvaptan hypersensitivity. Several limitations of this study with regards to translation of *in-vitro* findings to clinical manifestation are noted. Firstly, whilst the detected cross-reactivity with mozavaptan (and conversely, lack of cross reactivity with conivaptan and lixivaptan) was identified across multiple T-cell cell clones derived from multiple donors. This may not be the case in all individuals due to the heterogeneity of inter-individual immunological synapse conformations and thus different tolerances to structural diversion from tolvaptan itself required for T-cell activation. Secondly, whilst no cross reactivity was observed with DM-4107 responsive T-cell clones to the vaptans investigated. The possibility of analogous metabolites being formed from those vaptans, namely through azepine ring opening with the other compounds is not addressed here due to the limited metabolising capacity of peripheral blood mononuclear cells (PBMC) based *in-vitro* culture platforms. In future studies, we will endeavour to address aforementioned issues and continue to contribute to the understanding and development of vaptan safety within autosomal dominant polycystic kidney disease.

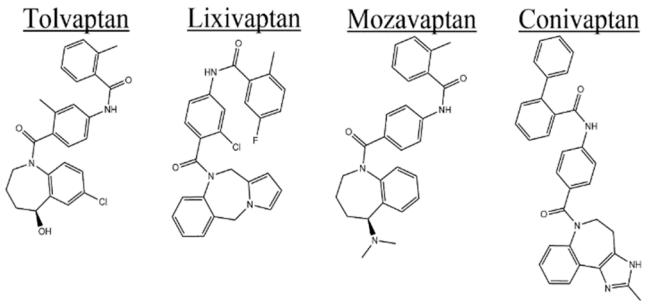

Fig. 1. Structure of the study drugs.

5. Conclusions

The index data demonstrates that Tolvaptan responsive T-cells can be capable of responding to structurally related compounds to elicit potentially deleterious T-cell responses. This is an important consideration with regards to drug development of second line therapeutic options for ADPKD, and for clinical management of who may be hypersensitive to tolvaptan.

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Author contributions

Sean Hammond conducted the experiments. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dean Naisbitt reports financial support was provided by Otsuka Pharmaceutical Co Ltd.

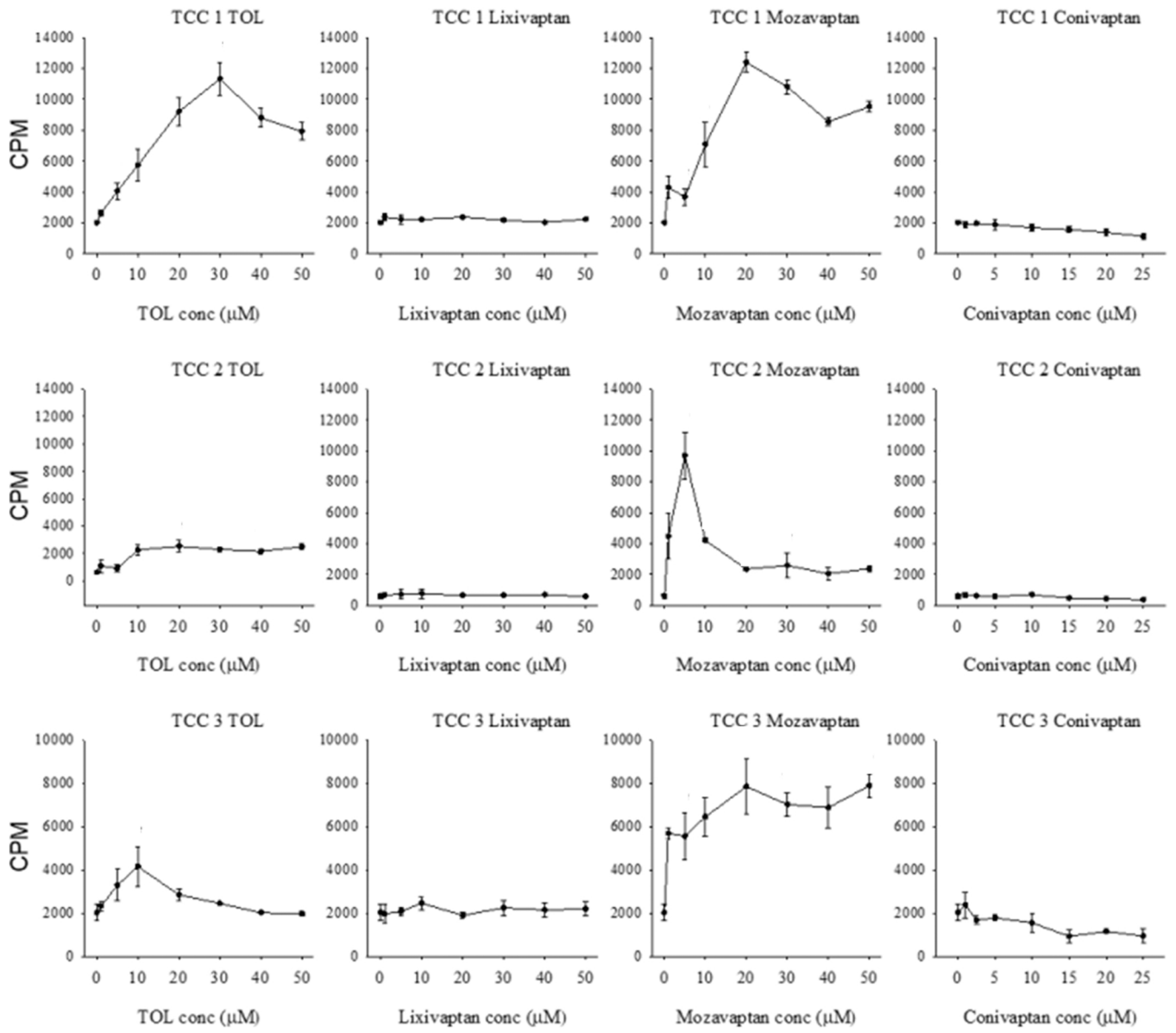

Fig. 2. Tolvaptan-responsive T-cell clones are cross reactive to mozavaptan, but not lixivaptan or conivaptan. Dose-response/cross reactivity series for three tolvaptan-responsive T-cell clones (TCC) across the four vaptans. T-cell clones (5×10^4) were cultured with autologous irradiated EBV-transformed B-cells (1×10^4) (96-well U bottomed) and indicated concentrations of tolvaptan (TOL), lixivaptan, mozavaptan, and conivaptan for 48 h. Data presented as mean + /- standard deviation counts per minute (CPM), a function of T-cell [^{3}H] thymidine incorporation with all experimental conditions performed using triplicate cultures.

Data Availability

No data was used for the research described in the article.

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