INTRODUCTION

- Translational experimental autoimmune encephalomyelitis (EAE) murine models have been used to assess peripheral and central activities of B- and T-cell-targeting multiple sclerosis (MS) drugs, without robust species cross-reactivity. 1

- Marmoset genetic and immunological profiles are comparable to those of humans which makes the EAE marmoset model particularly useful for research into the immunopathogenetic mechanisms of new therapies for MS. 1

- Cladribine is phosphorylated to the active compound by the enzyme deoxycytidine kinase. High levels of 2-deoxycytidine (2dC) levels can compete with cladribine and may reduce its in vivo efficacy. 2

- Here we present the feasibility of using an EAE marmoset model to assess selective depletion of lymphocytes subsets in blood, lymphoid organs and the central nervous system following cladribine exposure.

OBJECTIVES

- The aim of this study was to assess the effect of cladribine on marmoset lymphocyte survival and proliferation in vitro.

- The effect of cladribine on viability of freshly isolated, naïve or mitogen-stimulated marmoset lymphocytes.

- The viability of marmoset axillary lymph node cells taken from EAE marmosets.

- The effect of cladribine on proliferation of marmoset lymphocytes stimulated with PHA or ConA.

METHODS

- Following exposure to cladribine (1 nM–100 μM), in vitro proliferation and survival of naïve and activated (concanavalin A [ConA], phytomyrtagnin [PHA], MOG35-55, recombinant [rMOG] peripheral blood mononuclear cells [PBMCs]), spleen and lymph node mononuclear cells and EBV-infected B-cells were assessed.

- Cells were cultured for 24, 48 or 72 hours before analysis of lymphocyte proliferation and survival. 5

- For proliferation assays, cells were stimulated for 48 hours following incubation for 16 hours with 3H-thymidine to measure proliferation.

- The results were expressed as a stimulation index, calculated by dividing the average counts per minute (cpm) of stimulated cells by the average cpm of unstimulated cells. A stimulation index above 2 was set as cut-off for a positive proliferative response.

- The effect of cladribine on viability was assessed by flow cytometry after staining with the Fixable Viability Dye eFluor 506.

- Marmoset 2dC levels were measured by reverse-phase chromatography.

RESULTS

Survival Assays

- The effect of cladribine on viability of freshly isolated, naïve or mitogen (PHA, ConA) or MOG-stimulated marmoset PBMCs was studied at 24 and 48h.

- Cladribine reduces CD20+ B-cell and CD3+ T-cell viability, with a more pronounced effect on CD20+ B-cells (Figure 1, n=1).

- Marmoset blood levels of 2dC were measured by reverse-phase chromatography.

- The effect of cladribine on the viability of marmoset axillary lymph node (ALN) cells, stimulated with mitogen or antigen was studied after 72 hours by flow cytometry. Cells were gated on single cells, lymphocytes, CD20– versus CD20+ and then for CD20+ within the CD3+ compartment (Figure 2, n=2).

- EBV-infected B-cell (50% survival) was unaffected by cladribine at 24, 48 and 72 hours (Figure 3, n=2).

Proliferation Assays

- A dose-dependent inhibitory effect of cladribine was seen on proliferation of fresh PBMC stimulated for 72 hours with PHA or ConA. In the last 16 hours, 3H-thymidine was added to the culture to measure proliferation in the last 4 hours.

- No difference in effect of cladribine was seen between naïve versus stimulated cells.

- A gradual decline of proliferation was observed in the spleen and ALN cells of EAE marmoset mice re-stimulated with MOG35-55 and rMOG for 72 hours. Cladribine concentrations between 0.5–500 nM were used (Figure 4, n=1).

- The effect of cladribine on proliferation of spleen and axillary lymph node cells taken from EAE marmosets.

Figure 1. Effect of cladribine on the viability of freshly isolated, naïve or mitogen-stimulated marmoset PBMCs.

Figure 2. Effect of cladribine exposure on the viability of EAE marmoset lymph node cells.

Figure 3. EBV-infected B-cell survival.

Figure 4. Proliferation of naïve and mitogen-stimulated PBMCs following cladribine exposure.

Figure 5. Effect of cladribine on proliferation of spleen and axillary lymph node cells taken from EAE marmoset.

Figure 6. Effect of cladribine on proliferation of EBV-infected spleen or axillary lymph node cells.

CONCLUSIONS

- This study describes the in vitro efficacy of cladribine on marmoset lymphocyte proliferation and survival and the levels of 2dC in marmoset plasma.

- Cladribine-induced cell death was more pronounced for B-cells (100 nM to 1 μM) than for T-cells.

- No difference in the effect of cladribine was observed between naïve and mitogen-stimulated PBMCs.

- Cladribine inhibited proliferation and survival of EAE marmoset splen and axillary lymph node mononuclear cells after antigen re-stimulation.

- Cladribine did not affect survival of EBV infected marmoset cells but inhibited proliferation in a dose-dependent manner.

- In marmosets the highest blood 2dC value was 0.59 μM, therefore the low level of 2dC suggests that the marmoset model is appropriate for further study for PK/PD evaluation.

REFERENCES


ACKNOWLEDGEMENTS

This study was sponsored by EMD Serono Inc, a division of Merck KGaA, Darmstadt, Germany. Callithrix jacchus, a species of Callithrix family Callithrichidae, native to South America. This study was funded by Merck KGaA, Darmstadt, Germany. Merck KGaA is a business of EMD Serono Research & Development Inc, a business of Merck KGaA, Darmstadt, Germany. This abstract does not include any conflict of interest.

DISCLOSURES

No author has any proprietary or institutional conflicts of interest. None of the authors have any proprietary or institutional conflicts of interest.

Table 1. 2dC levels in marmoset blood

<table>
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<tr>
<th>Marmoset #</th>
<th>Sex</th>
<th>2dC (ng/mL)</th>
<th>2dC (µM)</th>
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<tbody>
<tr>
<td>ME381</td>
<td>Female</td>
<td>46.3±0.7</td>
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<tr>
<td>ME577</td>
<td>Female</td>
<td>26.8±0.1</td>
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<td>ME287</td>
<td>Female</td>
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<td>M10172</td>
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<tr>
<td>M10210</td>
<td>Male</td>
<td>74.5±0.5</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Note: mean±SEM of 2dC levels in marmoset plasma.

2dC levels suitable for PK/PD evaluation.

2dC: 2-deoxycytidine.