Effects of cladribine on proliferation, survival and cytokine release of human astrocytes

Authors: Herena Eixarch[1], Laura Calvo-Barreiro[1], Nicolás Fissolo[1], Ursula Boschert[2], Manuel Comabella[1], Xavier Montalban[1], Carmen Espejo[1]

[1]Servei de Neurologia-Neuroimmunologia. Centre d'Esclerosi Múltiple de Catalunya (Cemcat). Institut de Recerca Vall d'Hebron (VHIR). Hospital Universitari Vall d'Hebron. Universitat Autònoma de Barcelona. Barcelona, Spain.

[2]Ares Trading S.A., Eysins, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany.

Disclosures and Funding sources

DISCLOSURES:

- **H Eixarch**, **L Calvo-Barreiro**, **N Fissolo** and **C Espejo** have nothing to disclose
- **U Boschert** is an employee of Ares Trading S.A., Eysins, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany.
- **M Comabella** has received compensation for consulting services and speaking honoraria from Bayer Schering Pharma, Merck KGaA, Darmstadt, Germany, Biogen-Idec, Teva Pharmaceuticals, Sanofi-Aventis, Genzyme, and Novartis
- **X Montalban** received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Actelion, Alexion, Bayer, Biogen, Celgene, EMD Serono, Genzyme, Immunic, Medday, Merck KGaA, Darmstadt, Germany, Mylan, Nervgen, Novartis, Roche, Sanofi-Genzyme, Teva Pharmaceutical, TG Therapeutics, Excemed, MSIF and NMSS

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Introduction and Purpose

- Cladribine is a synthetic purine nucleoside analogue that has demonstrated beneficial effects in patients with relapsing-remitting multiple sclerosis (MS).

- Cladribine is phosphorylated inside the cell by deoxycytidine kinase (DCK) to its active form 2-chloro-2-deoxy-β-D-adenosine triphosphate (2-CdATP).

- Accumulation of 2-CdATP triggers apoptosis in both dividing and quiescent lymphocytes via direct or indirect actions on DNA and mitochondria (*Lindemalm et al, 2004*).

- Cladribine may act as an immune-modulator and/or neuroprotector via adenosine receptor interaction (*Laugel et al, 2011*).

- Although the effects of cladribine on T and B cell survival and proliferation is well studied, it remains unknown if it affects immune functions of glial cell populations of the central nervous system.

The aim of the study was to test the effect of cladribine on proliferation, survival and cytokine release of human astrocytes.

Methods

Human astrocytes

isolated from cerebral cortex

(from ScienCell Research Laboratories)

1) Effect of cladribine on proliferation, cell survival, and immune status in a deoxycytidine kinase (DCK)-dependent manner

Culture conditions

1) No cladribine (*Basal*)
2) 0.002 µM† cladribine
3) 0.02 µM† cladribine
4) 0.2 µM* cladribine
5) 2 µM cladribine

† The 0.002 µM to 0.02 µM concentrations reflect rough estimated brain exposure levels of cladribine (*Liliemark, 1997*).

* The 0.2 µM concentration is in line with human blood C_{max} /steady state cladribine levels measured in clinical trials (*Hermann et al, 2019*).

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Methods

Human astrocytes

isolated from cerebral cortex

(from ScienCell Research Laboratories)

2) Effect of cladribine on proliferation, cell survival, and immune status in a DCK-independent manner

Culture conditions

1) Deoxycytidine* 100 µM
2) Deoxycytidine 100 µM + 0.02 µM cladribine
3) Deoxycytidine 100 µM + 0.2 µM cladribine

* Deoxycytidine is the preferred substrate for DCK. Deoxycytidine was added 30 minutes before cladribine to astrocyte cultures in order to saturate DCK.

Statistical analysis

- Mixed models to account for repeated measures were performed.

- Multiple comparisons were performed with Dunnett's post-hoc test taking the *Basal* condition (survival and proliferation experiments) or *Stimulated* condition (immune cell function experiments) as the reference.

Methods

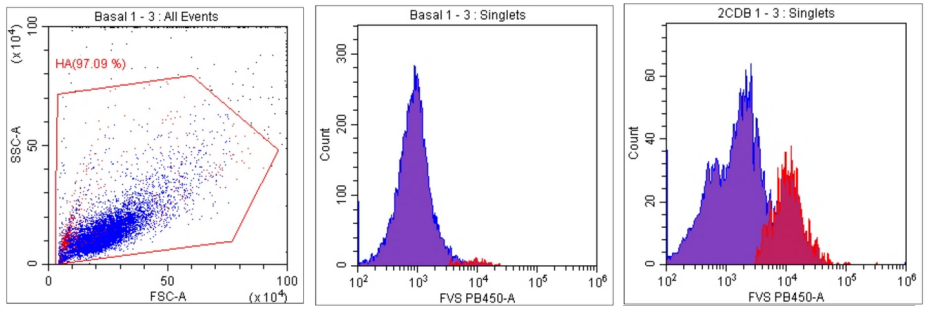
Human astrocytes

isolated from cerebral cortex

(from ScienCell Research Laboratories)

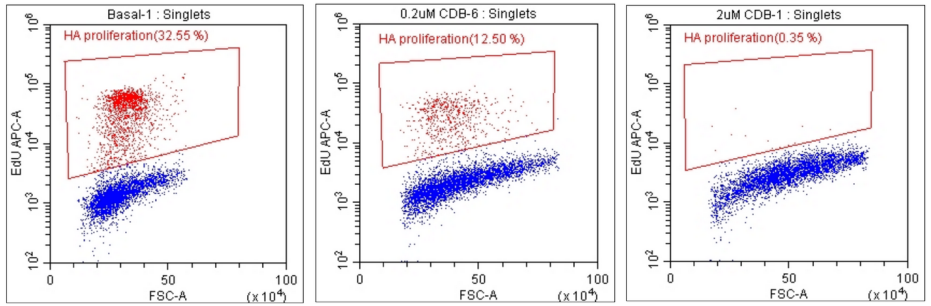
Cell death assessment:

- Normal cell growth for 72h (in the presence of cladribine +/- deoxycytidine)
- Harvesting of cells and viability staining
- Percentage of cell death (flow cytometry)



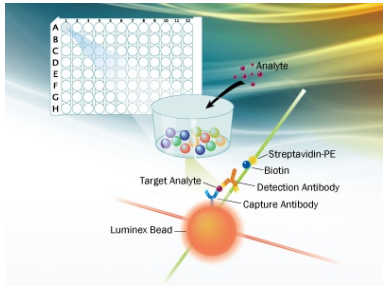
Proliferation assessment:

- Normal cell growth for 72h (in the presence of cladribine +/- deoxycytidine)
- EdU incorporation
- Harvesting of cells and EdU detection (chemical reaction + detection)
- Percentage of proliferating cells (flow cytometry)



Immune cell function assessment:

- 20ng/ml TNFα + 20ng/ml IL1β for 6h + normal cell growth for additional 18h
- Cladribine +/- deoxycytidine were added for the whole 24h of culture
- Collection and storage of supernatant
- Quantification of GM-CSF, TNF-α, IL-1β, IL-6 (Luminex technology)



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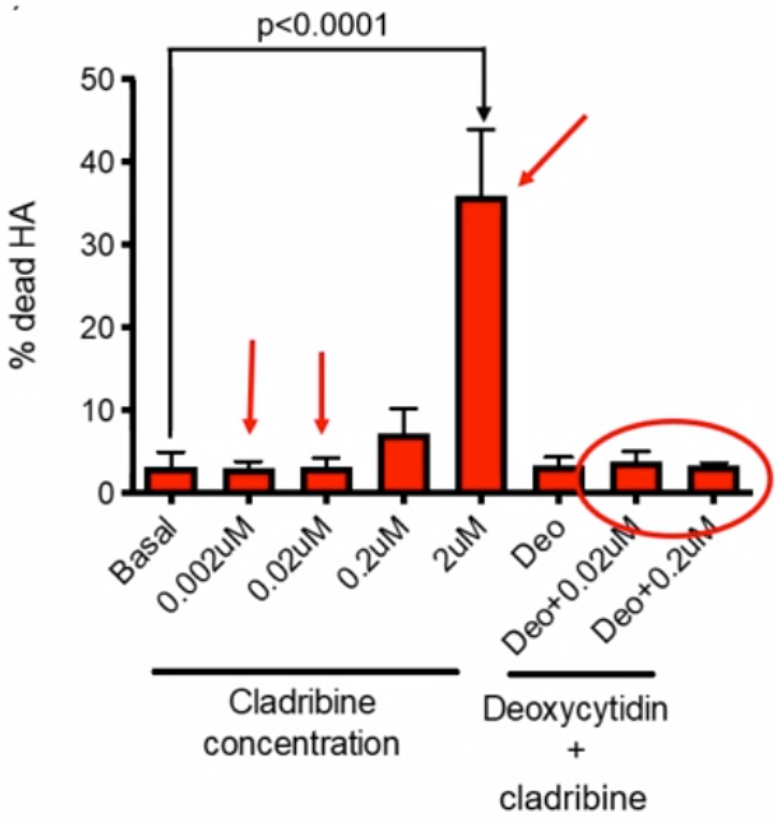
Results & Conclusions

The **mean estimated brain exposure to cladribine** does not influence **survival** of human astrocytes neither in a DCK-dependent nor in a DCK-independent manner.



- The highest concentration (2 µM) of cladribine reduced cell survival of human astrocytes.

- Lower concentration of cladribine (0.02-0.002 µM), reflecting estimated human MS brain exposure levels, did not affect human astrocyte survival of human astrocytes.

- Cladribine did not affect cell survival when the DCK pathway was saturated with the substrate deoxycytidine.

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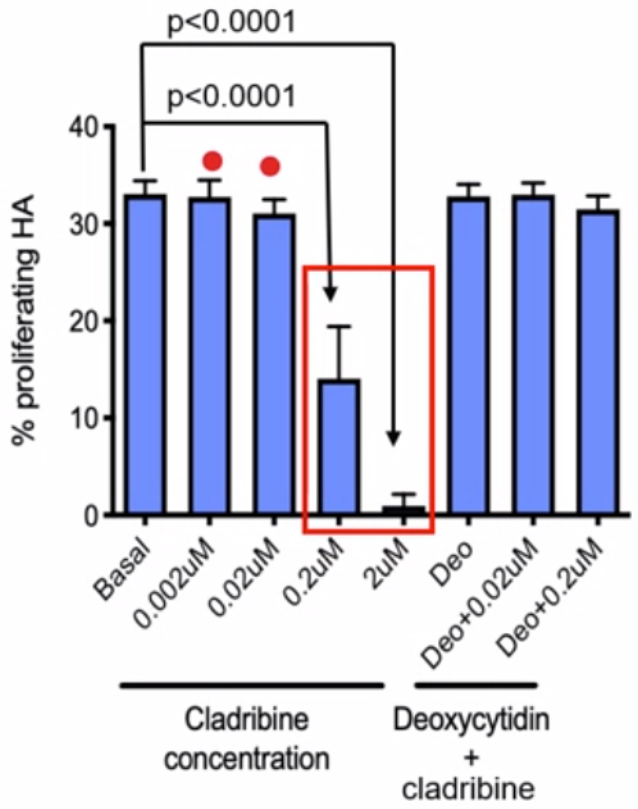
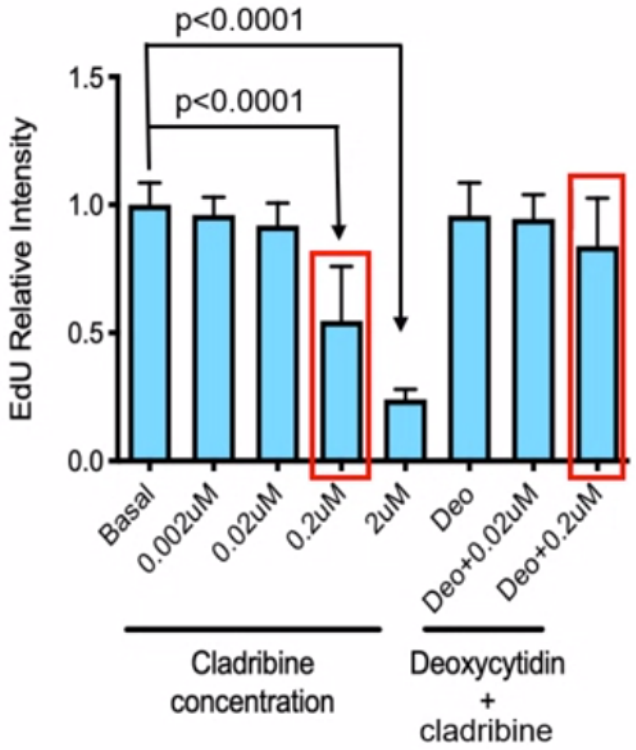
Experiments were run in duplicate. Data represent 5 independent experiments.
Data are represented as the mean±standard deviation.

Results & Conclusions

The **mean estimated brain exposure to cladribine** does not influence **proliferation** of human astrocytes neither in a DCK-dependent nor in a DCK-independent manner.





- High concentration (2, 0.2 µM) of cladribine reduced proliferation of human astrocytes.

- Lower concentration of cladribine (0.02-0.002 µM), reflecting estimated human MS brain exposure levels, did not affect human astrocyte proliferation of human astrocytes.

- Cladribine did not affect proliferation when the DCK pathway was saturated with the substrate deoxycytidine.

- Relative Intensity of EdU fluorescence respect to basal was reduced in astrocytes cultured with 0.2 µM cladribine, suggesting that exposure to 0.2 µM cladribine limited the capacity of proliferation of astrocytes.

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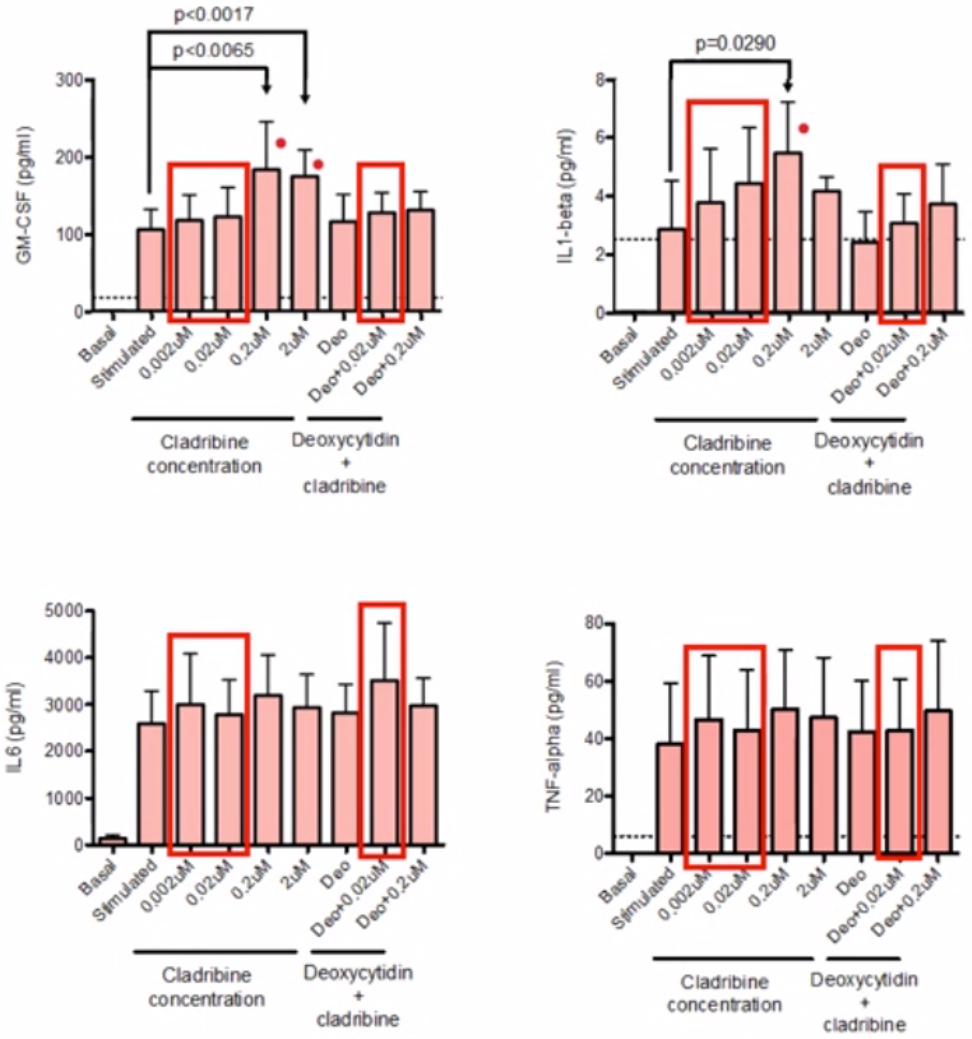
Experiments were run in duplicate. Data represent 5 independent experiments. Data are represented as the mean±standard deviation.

Results & Conclusions



The **mean estimated brain exposure to cladribine** does not alter the **cytokine-release pattern** of stimulated human astrocytes via DCK-dependent pathway or via DCK-independent pathway.

- High concentration (2, 0.2 µM) of cladribine enhanced GM-CSF secretion on cytokine-stimulated human astrocytes.

- 0.2 µM cladribine increased IL-1β secretion on cytokine-stimulated human astrocytes.

- Lower concentration of cladribine (0.02-0.002 µM), reflecting estimated human MS brain exposure levels, did not influence the cytokine-release pattern of cytokine-stimulated human astrocytes.

- Cladribine did not affect the immune cell function when the DCK pathway was saturated with the substrate deoxycytidine.

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Experiments were run in duplicate. Data represent 3 independent experiments.Data are represented as the mean±standard deviation.
The dotted line indicates the lower limit of quantification of the technique for each cytokine

Summary & Main Conclusion

- The **mean estimated brain exposure** to cladribine does not influence **survival or proliferation** of human astrocytes neither in a DCK-dependent nor in a DCK-independent manner.

- The **mean estimated brain exposure** to cladribine does not affect the **cytokine-release pattern** of stimulated human astrocytes via DCK-dependent pathway or via DCK-independent pathway.

Our data suggest that cladribine **does not affect** the normal human astrocyte function in MS patients.