Enantioselective Neuroprotective Effects of Tacripyrine ITH122 Against Oxygen and Glucose Deprivation in Rat Hippocampal Slices

Alejandro Romero,^{1,2,5} Vanessa Gomez-Rangel,^{1,2} Javier Egea,^{1,2,3} Abdelouahid Samadi,⁴ José Marco-Contelles⁴ & Manuela G. Lopez^{1,2,3}

¹ Instituto Teófilo Hernando, Universidad Autónoma de Madrid, Madrid, Spain

² Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

³ Instituto de Investigación Sanitaria, Hospital Universitario la Princesa, Universidad Autónoma de Madrid, Madrid, Spain

⁴ Laboratorio de Química Médica (IQOG, CSIC), Madrid, Spain

⁵ Current address: Departamento de Toxicología y Farmacología, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain

Correspondence

Dr. Alejandro Romero, Departamento de Toxicología y Farmacología, Facultad de Veterinaria, Universidad Complutense de Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain.

Tel.: +34-913943834;

Fax: +34-913943840;

E-mail: manarome@ucm.es

Received 13 January 2013; revision 18

January 2013; accepted 20 January 2013.

doi: 10.1111/cns.12073

Stroke is a major cause of death and the leading cause of long-term disability in industrialized countries [1]. In the context of the complex pathophysiological mechanisms underlying cerebral ischemia, a line of research has focused on the reduction in oxidative stress after ischemia–reperfusion injury of the brain. Reactive oxygen species (ROS) generation and mitochondrial depolarization are known to be important in the initial injury involved with ischemia–reperfusion (I/R) [2,3].

We have previously designed and synthesized tacrine and 1,4-dihydropyridine hybrids (“tacripyrines”) whose biological properties led us to conclude that these compounds are promising new drugs for the treatment for Alzheimer’s disease [4,5]. ITH122 was one of the compounds that showed an interesting protective profile against oxidative stress. In this study, we have resolved the corresponding enantiomers of ITH122 ((*S*)-ITH122 and (*R*)-ITH122; Figure 1A) by chiral HPLC [6]. Then, we evaluated their neuroprotective effects in an *in vitro* model of cerebral ischemia. Rat hippocampal slices were subjected to 15-min oxygen-glucose deprivation (OGD) followed by a 2-h reoxygenation period (Reox) (see protocol in Figure 1B). The neuroprotective effects of racemic ITH122, (*S*)-ITH122, and (*R*)-ITH122 in rat hippocampal slices subjected to OGD/Reox were evaluated at the concentrations of 0.3–10 μ M. Cell viability of hippocampal slices was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-

mide (MTT) assay. To measure the generation of ROS, we used the molecular probe H₂DCFDA. TMRE was also employed to evaluate mitochondrial membrane potential ($\Delta\Psi$ m) in hippocampal slices for the estimation of the mitochondrial function. Statistical comparisons between experimental and control groups were performed by one-way ANOVA followed by Newman–Keuls post hoc test.

Cell viability, normalized to 100% of control slices, was reduced significantly after OGD/Reox (Black column in Figure 1C–E), and maximum protection with racemic ITH122 was observed at 3 μ M (Figure 1C). While enantiomer (*S*)-ITH122 offered a concentration-dependent protection against OGD/Reox-induced cell death in hippocampal slices (Figure 1E), enantiomer (*R*)-ITH122 failed to protect at any of the concentrations tested (Figure 1D). Because ROS production and the loss of $\Delta\Psi$ m have a pathophysiological significance in ischemic stroke followed by reperfusion [2,7], we evaluated whether enantiomer (*S*)-ITH122 (3 μ M) could modify these parameters. Indeed, enantiomer (*S*)-ITH122 was able to reduce ROS production to control levels (Figure 2D) and restored $\Delta\Psi$ m (Figure 2H) induced by OGD/Reox.

In conclusion, enantiomer (*S*)-ITH122 is responsible for the neuroprotective activity of the racemic ITH122, while (*R*)-ITH122 is ineffective. Although the underlying mechanisms remain elusive, these findings highlight the importance of the absolute

Figure 1 (A) Structures of (S)- and (R)-ITH122. (B) Experimental protocol; Slices were subjected to 15-min OGD followed by 2-h reoxygenation in the absence (OGD) or presence of 0.3–10 μM racemic ITH122 and the two enantiomers. (C) Racemic ITH122 and (D) (R)-ITH122 increase cell viability of rat hippocampal slices subjected to OGD/Reox. (E) (S)-ITH122 not modified cell viability. (C–E) Shows hippocampal viability measured by MTT. Data are means $\pm$ SEM of 6 independent experiments. $^{##}P < 0.01$ and $^{###}P < 0.001$ with respect to control slices; $^{**}P < 0.01$ and $^{***}P < 0.001$ with respect to slices subjected to OGD/Reox in the absence of drugs.

configuration at one stereocenter of the selected tacipyryne ITH122 in controlling its biological activity. Furthermore, they can provide new insights into the development of therapeutic agents for the intervention of ischemia–reperfusion injury.

Acknowledgments

We thank the Spanish Ministry of Economy and Competence grant No. SAF2009-07271 to JMC and No. SAF2009-12150 and SAF2012-32223 to MGL. We also thank the continued support of Fundación Teófilo Hernando.

References

- Balami JS, Chen RL, Grunwald IQ, Buchan AM. Neurological complications of acute ischaemic stroke. *Lancet Neurol* 2011;10:357–371.
- Crack PJ, Taylor JM. Reactive oxygen species and the modulation of stroke. *Free Radic Biol Med* 2005;38:1433–1444.
- Abramov AY, Scorziello A, Duchon MR. Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. *J Neurosci* 2007;27:1129–1138.
- Marco-Contelles J, Leon R, De Los Rios C, et al. Tacipyrynes, the first tacrine-dihydropyridine hybrids, as multitarget-directed ligands for the treatment of

Figure 2 The top part of the figure illustrates DCFDA fluorescence microphotographs of hippocampal slices under experimental conditions (A–C). (D) (S)-ITH122 (3 μM) reduces the level of ROS induced by OGD/Reox. The bottom part illustrates TMRE fluorescence (E–G). (H) (S)-ITH122 (3 μM) reduces significantly mitochondrial depolarization in hippocampal slices subjected to OGD/Reox. Data are means $\pm$ SEM of 6 independent experiments. $^{\#}P < 0.05$ and $^{##}P < 0.01$ with respect to control; $^{*}P < 0.05$ in comparison with OGD/Reox alone.

Conflict of interest

The authors declare no conflict of interest.

- Alzheimer's disease. *J Med Chem* 2009;**52**: 2724–2732.
5. Marco-Contelles J, Leon R, De Los Rios C, et al. Novel multipotent tacrine-dihydropyridine hybrids with improved acetylcholinesterase inhibitory and neuroprotective activities as potential drugs for the treatment of Alzheimer's disease. *J Med Chem* 2006;**49**:7607–7610.
6. Bartolini M, Pistozzi M, Andrisano V, et al. Chemical and pharmacological studies on enantiomerically pure p-methoxytacripyrines, promising multi-target-directed ligands for the treatment of Alzheimer's disease. *ChemMedChem* 2011;**6**:1990–1997.
7. Iijima T. Mitochondrial membrane potential and ischemic neuronal death. *Neurosci Res* 2006;**55**:234–243.