J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

Molecular mechanisms mediating the neuroprotective effects of quinacrine and minocycline on cell death induced by the prion protein fragment 90-231 (hPrP90-231)

V. Villa[1], A. Corsaro[1], S. Thellung[1], A. Simi[1], M. Nizzari[1], M. Tonelli[2], V. Boido[2], A. Aceto[3], T. Florio[1]*

[1] Section of Pharmacology, Dept. Internal Medicine, University of Genova, Viale Benedetto XV 2, 16132 Genova, Italy
[2] Dept. Pharmaceutical Sciences, University of Genova, Viale Benedetto XV 3, 16132, Genova, Italy
[3] Dept. of Biomedical Sciences, University of Chieti, Via dei Vestini 31, 66013 Chieti, Italy
* tullio.florio@unige.it

KEY WORDS: prion protein, quinacrine, minocycline

Abstract

The effects of quinacrine and minocycline on the toxicity induced by hPrP90–231 were studied. By mild thermal denaturation, hPrP90–231 can be converted in a toxic PrP^{Sc}-like structure affecting the survival of SH-SY5Y cells. Quinacrine and minocycline prevented hPrP90–231-induced toxicity interfering with different mechanisms: protective effects of quinacrine are mediated by the binding to the fragment that abolished hPrP90–231 structural changes and cell internalization, whereas, minocycline reverted MAP kinase neurotoxic signaling exerted by the prion fragment.

Introduction

PrP misfolding represents the causative event of brain accumulation of PrP^{Sc} and neuronal death during prion diseases. Different approaches to face up prion diseases have been pursued and the chemotherapeutic approach seems yet to be the most promising. In this study we evaluated the potential neuroprotective effects of two different classes of drugs on the neurotoxicity induced by hPrP90–231 corresponding to the protease-resistant core of PrP^{Sc} identified in prion-infected brains. We studied the effects of quinacrine, an antimalaric drug that was shown as potent antiprion compound *in vitro*, and minocycline, a tetracycline with known neuroprotective activity.
In our experimental model, mild thermal denaturation (1h at 53°C) converts hPrP90–231 from a native PrP^{C}-like conformation into a PrP^{Sc}-like structure. In virtue of these alterations, hPrP90–231 powerfully affects SH-SY5Y cells survival activating pro-apoptotic pathways, while in the native a-helix-rich conformation it is not toxic for the cells.

Materials and Methods

SH-SY5Y human neuroblastoma cell line was used as cell model in consideration of its sensitivity to PrP derived peptides All treatments were performed in culture medium supplemented with 2% FBS to inhibit cell proliferation without inducing a significant reduction in cell viability [1]. Quantification of cell viability was performed by MTT reduction test. Immunoblotting and binding analysis were performed as in previous studies [2, 3].

Results

After thermal denaturation, hPrP90-231 treatment induced significant reduction of SH-SY5Y viability, an effect completely abolished when hPrP90-231 thermal treatment was carried in the presence of quinacrine (1μM). Quinacrine did not significantly affect cell viability by itself. Conversely, the treatment with quinacrine failed to prevent cell death when hPrP90-231 was already converted in the toxic conformation. Quinacrine binds to hPrP90-231 with high affinity (K_D=0,49mM) and prevents PrP^{Sc}-like conformational changes responsible of the neurotoxicity induced by thermal denaturation of the peptide. Mild thermal denaturation also renders hPrP90-231 partially resistant to PK proteolysis [2] due to changes in conformation and/or aggregation state. This property is thought, *in vivo*, to favor PrP^{Sc} accumulation in the brain, leading to the disease development. In agreement with previous studies [2, 3], the α-helix structured peptide was sensitive to PK treatment, but, upon conversion in a β-sheet rich conformer, hPrP90-231 showed partial resistance (about 40% of the protein input). In the presence of quinacrine, a significant reduction of the PK resistance of the toxic conformer of hPrP90-231 was observed, confirming the effects of this drug on hPrP90-231 secondary structure.
Also the treatment with minocycline (1μM) was able to completely counteract cell death induced by toxic hPrP90–231. Performing experiments to assess the binding

properties toward hPrP90-231, we show that the binding of minocycline (K_D=2.648 mM) displayed low affinity. By immunoblotting experiments, we show that hPrP90–231-induced apoptosis is dependent on the inhibition of ERK1/2 responsiveness to neurotrophic factors, removing a tonic inhibition of p38 activity that, in turn, leads to caspase 3 activation. Treatments with minocycline prevented hPrP90–231-induced toxicity interfering with this mechanism while hPrP90–231 structural changes or cell internalization were not affected.

Discussion

Quinacrine and minocycline prevent hPrP90–231-induced toxicity interfering with different mechanism: quinacrine effects are mediated by the binding to the PrP fragment preventing hPrP90–231 conformational gain of toxicity, whereas, minocycline reverts the intracellular pro-apoptotic signaling induced by the peptide. These results highlight the different pharmacological modality by which prion toxicity can be affected and support the use of recombinant hPrP90-231 as a tool for preclinical screening of novel molecules to be used in PrP^{Sc}-dependent neurodegeneration.

Grants: MiUR-PRIN 2008 and Compagnia San Paolo 2005.1411 to TF.

References

[1] Corsaro A., Thellung S., Villa V., Principe D.R., Paludi D., Arena S., Millo E., Damonte G., Aceto A., Florio T. 2003. Prion protein fragment 106-126 induces a p38 MAP kinase-dependent apoptosis in SH-SY5Y neuroblastoma cells independently from the amyloid fibril formation. Ann. N.Y. Acad. Sci., 1010: 610-622

[2] Corsaro A., Thellung S., Chiovitti K., Villa V., Simi A., Raggi F., Paludi D., Russo C., Aceto A., Florio T. 2009. Dual modulation of ERK1/2 and p38 MAP kinase activities induced by minocycline reverses the neurotoxic effects of the prion protein fragment 90-231. Neurotox. Res., 15: 138-154.

[3] Villa V., Tonelli M., Thellung S., Corsaro A., Tasso B., Novelli F., Canu C., Pino A., Chiovitti K., Paludi D., Russo C., Sparatore A., Aceto A., Boido V., Sparatore F., Florio T. 2011. Efficacy of Novel Acridine Derivatives in the Inhibition of hPrP90-231 Prion Protein Fragment Toxicity. Neurotox. Res., 19: 556-574