In vitro and *in silico* analysis of the vascular effects of asymmetrical *N,N*-bis(alkanol)amine aryl esters, novel multidrug-resistance reverting agents

M. Durante[1], F. Fusi[1], O. Spiga[2], A. Trezza[2], M. Frosini[1], E. Floriddia[3], E. Teodori[3], S. Dei[3], S. Saponara[1]
1 Dipartimento di Scienze della Vita, Università degli Studi di Siena, via A. Moro 2, 53100 Siena (Italy)
2 Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, via A. Moro 2, 53100 Siena (Italy)
3 Dipartimento NEUROFARBA-Sezione di Farmaceutica e Nutraceutica, Università di Firenze, via Ugo Schiff 6, 50019 Sesto Fiorentino (Italy)

Purpose Asymmetrical N,N-bis(alkanol)amine aryl esters (FRA77, GDE6, and GDE19) are potent multidrug-resistance (MDR) reversers (Dei et al. 2014). Their structures loosely remind that of the Ca^{2+} antagonist verapamil. Therefore, the aim of this study was to investigate their vascular activity *in vitro*.
Methods Their effects on the mechanical activity of fresh and cultured rat aorta rings, on $Ca_v1.2$ channel current ($I_{Ca1.2}$) of A7r5 cells (Fusi et al. 2008; Murata et al. 2001) and of their cytotoxicity on A7r5 and EA.hy926 cells (Brizi et al. 2015) were analyzed. Docking at the rat $\hat{I}\pm_{1C}$ subunit of the $Ca_v1.2$ channel was simulated *in silico* (Saponara et al. 2016).
Results Compounds tested were cytotoxic at concentrations > 1 μM (FRA77, GDE6, GDE19) and > 10 μM (verapamil) in EA.hy926 cells, or > 10 μM (FRA77, GDE6, GDE19) and at 100 μM (verapamil) in A7r5 cells. In fresh rings, the three compounds partly antagonized phenylephrine and 60 mM K^+ (K60)-induced contraction at concentrations ≥ 1 μM and ≥ 3 μM, respectively. On the contrary, verapamil fully relaxed rings pre-contracted with both agents. In cultured rings, 10 μM GDE6, GDE19, FRA77 and verapamil significantly reduced the contractile response to both phenylephrine and K60. Similarly to verapamil, the three compounds docked at the $\hat{I}\pm_{1C}$ subunit, interacting with the same amino acids residues. FRA77, GDE6 and GDE19 inhibited $I_{Ca1.2}$ with IC_{50} values one order of magnitude higher than that of verapamil.
Conclusions FRA77-, GDE6-, and GDE19-induced vascular effects occurred at concentrations that are at least one order of magnitude higher than those effectively reverting MDR. Though an unambiguous divergence between MDR reverting and vascular activity is of overwhelming importance, these findings consistently contribute to the design and synthesis of novel and potent chemosensitizers.
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