Characterization of mesoridazine transport in human cerebral microvessel endothelial cells, hCMEC/D3

Yasuyuki Debori[1], Tomoko Igari[1], Masanori Nakakariya[1], Nobuyuki Amano[2], Yuma Tega[1], Kei Higuchi[1], Takashi Okura[1] and Yoshiharu Deguchi[1]

1.Laboratory of Drug Disposition and Pharmacokinetics, Faculty of Pharma-Sciences, Teikyo University
2.Drug Disposition and Analysis, Research Division, Axcelead Drug Discovery Partners, Inc.

Purpose

Mesoridazine is a cationic drug and known to be distributed to brain ($K_{p,uu}$ = 0.4 in human) despite a substrate of MDR1. This suggests that some uptake transporters would be involved in the brain uptake process of mesoridazine in human.

In present study, we focused on the uptake process of mesoridazine into brain and aimed to characterize the mesoridazine transport using human cerebral microvessel endothelial cell line, hCMEC/D3 cells.

Materials and Methods

Mesoridazine uptake into hCMEC/D3 cells was measured under various conditions to evaluate the time-, temperature-, concentration-, energy- and ion-dependencies. Inhibition study was also performed with selected organic cations. Mesoridazine was quantified by LC-MS/MS system, Nexera XR (Shimadzu) and TQ4500 (Sciex). Multiple reaction monitoring transition was observed at Q1/Q3 387.16/126.1 for mesoridazine.

Results

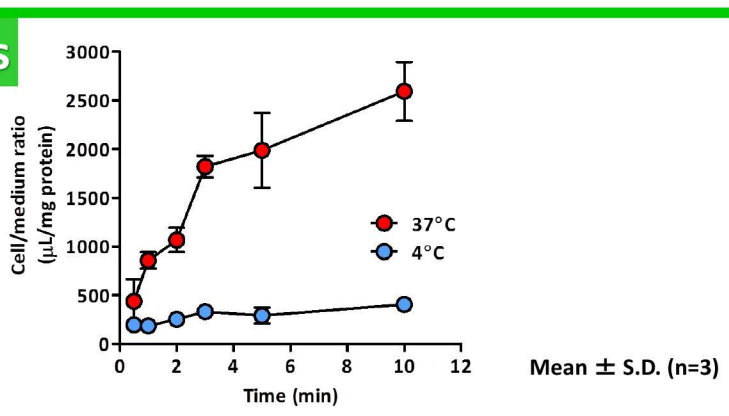

Fig. 1 Time profile of mesoridazine (1 μM) uptake by hCMEC/D3 cells

Uptake of mesoridazine in hCMEC/D3 cells was observed in time- and temperature-dependent manner. This result indicated that carrier-mediated transport mechanism was involved in the uptake of mesoridazine in hCMEC/D3 cells.

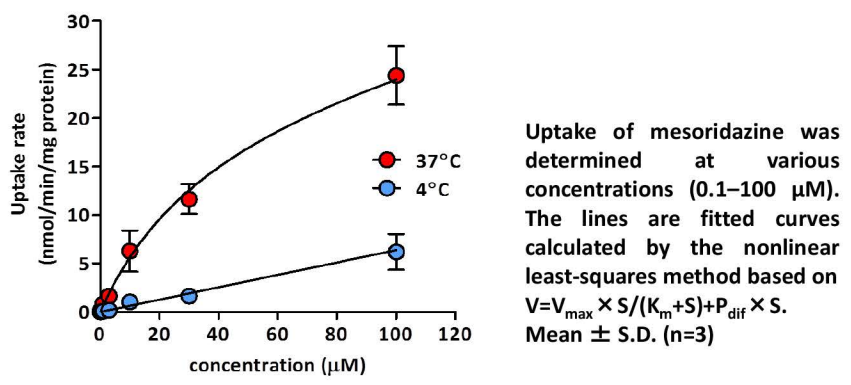

Fig. 2 Concentration-dependent uptake of mesoridazine by hCMEC/D3 cells

The concentration dependence of the uptake of mesoridazine can be explained by one saturable and one non-saturable component in hCMEC/D3, with K_m, V_{max}, and P_{dif} values of 38.9 μM, 24.4 nmol/min/mg protein, and 63.9 μL/min/mg protein, respectively.

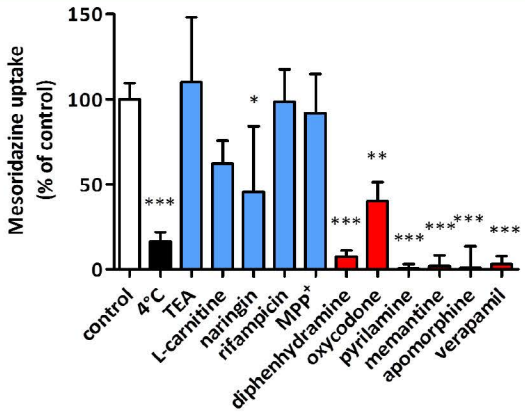

Fig. 3 Inhibitory effect of various compounds on mesoridazine uptake by hCMEC/D3 cells

The uptake of mesoridazine was significantly inhibited by typical cationic compounds, but not significantly inhibited by carnitine (a substrate and inhibitor of OCTN2), TEA and MPP^+ (typical substrate and/or inhibitor of OCTs), or rifampicin (a typical inhibitor of OATPs). This result suggests that transporter(s) different from the known cation transporters are involved in the uptake of mesoridazine in hCMEC/D3 cells.

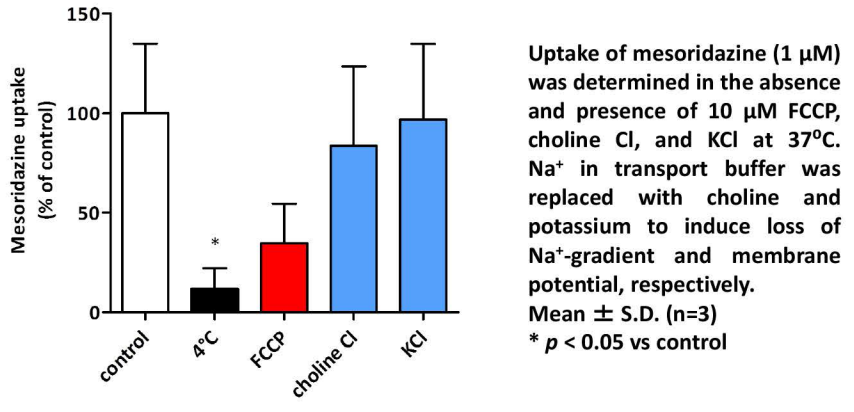

Fig. 4 Effects of proton gradient, extracellular Na^+, and membrane potential on mesoridazine uptake by hCMEC/D3 cells

Mesoridazine uptake was moderately decreased by FCCP, a protonophore, but not by replacement of extracellular sodium ion with choline and potassium, suggesting that H^+-dependent, Na^+-independent, and membrane potential-independent transporter, is involved in the uptake of mesoridazine.

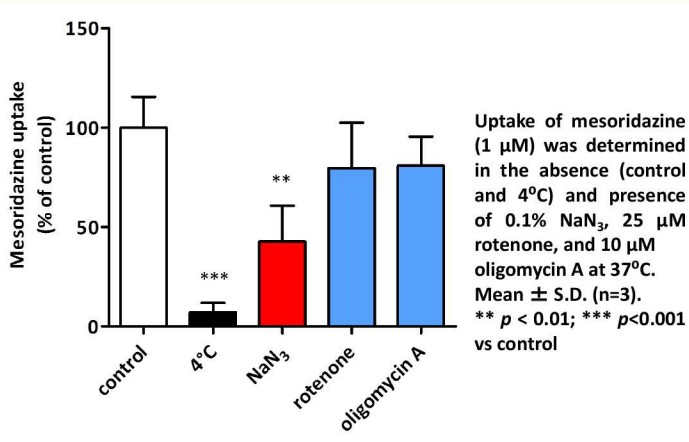

Fig. 5 Effects of metabolic inhibitors on mesoridazine uptake by hCMEC/D3 cells

The uptake of mesoridazine was inhibited by 50 % by pretreatment with sodium azide. These results suggest that the uptake process of mesoridazine is energy-dependent.

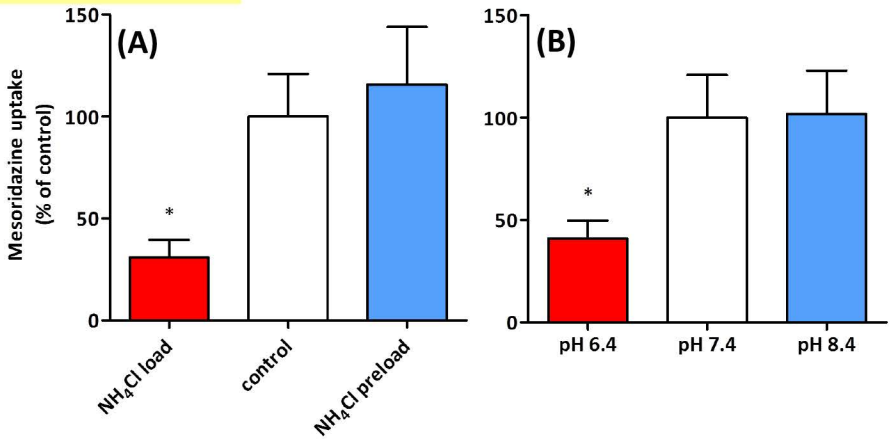

Fig. 6 Effects of intracellular (A) and extracellular (B) pH on mesoridazine uptake by hCMEC/D3 cells

NH_4Cl load (pH_i: ↑) reduced significantly mesoridazine uptake. In contrast, no significant changes in mesoridazine uptake were observed by NH_4Cl preload (pH_i: ↓). This unexpected characteristic was supported by the result that mesoridazine uptake showed no significant difference between extracellular pHs of 7.4 and 8.4, suggesting existence of pH-insensitive uptake mechanism in alkaline pH range (> pH 7.4).

Conclusion

The present study suggested that not only proton-coupled organic cation antiporter similar to the pyrilamine transporter, but also other transport system may be involved in the uptake process of mesoridazine into hCMEC/D3 cells.

Acknowledgement

We thank Dr. Hideki Hirabayashi (Takeda Pharmaceutical Company Limited) for helpful discussion.

COI disclosure information

We have no financial relationship to disclose for our presentation contents.