[Purpose] Several quinolones undergo the biotransformation to their sulfamates before excretion. In the present study, we have investigated the N-sulfoconjugation of quinolones and other amine drugs (ciprofloxacin, moxifloxacin, garenoxacin, desipramine and metoclopramide) to assess the contribution of specific human cytosolic sulfotransferases (SULTs) to the reactions.

[Methods] The sulfations were assessed using purified human (h) recombinant SULTs (hSULT1A1, hSULT1A3, hSULT1B1, hSULT1C1, hSULT1E1 and hSULT2A1) and human liver cytosols (HLCs).

[Results and Discussion] Among the enzymes examined, hSULT2A1 exhibited N-sulfating activities toward all drugs tested, whereas the other five different forms showed no detectable activities except hSULT1A1 for garenoxacin sulfation. Sulfating activity of hSULT2A1 was the highest toward moxifloxacin (6.3 ± 0.1 nmol/min/mg protein) at the substrate concentration of 100 µM. Kinetic analyses demonstrated that HLC-mediated N-sulfation were monophasic for all the substrates examined with apparent $K_m$ values comparable to those mediated by hSULT2A1. $K_m$ values for N-sulfation mediated by hSULT2A1 were as follows: 1.08 ± 0.03 mM for ciprofloxacin, 0.53 ± 0.01 mM for moxifloxacin, 0.19 ± 0.01 mM for garenoxacin, 0.054 ± 0.001 mM for desipramine and 2.32 ± 0.12 mM for metoclopramide. The sulfating activities of HLCs toward the amines were well correlated with those for O-sulfation of dehydroepiandrosterone, a hSULT2A1 probe substrate.

[Conclusion] The present results unequivocally demonstrate that hSULT2A1 is responsible for N-sulfation of quinolones and possibly for other therapeutic amine drugs in humans.

---

[2-B2-10-1]

POSSIBLE INVOLVEMENT OF MITOCHONDRIAL MEMBRANE-BOUND GLUTATHIONE TRANSFERASE (MTMGST1) IN PEROXYNITRITE-INDUCED MEMBRANE PERMEABILITY TRANSITION PORE OPENING

Naoki Imaizumi, Ryosuke Katayama, Enkhbaatar Ulziikhishig, Yoko Aniya
Laboratory of Molecular Genetics and Pharmacology, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan.

The membrane permeability transition (MPT) pore is a non-specific pore in the inner mitochondrial membrane which is a key step of apoptosis and/or necrosis, and is opened by various conditions including calcium overload and oxidative stress. However, the pore forming component and opening mechanism have not been understood precisely. In the present study we investigated a relationship between mtMGST1 activation and MPT pore opening by using peroxynitrite (ONOO$^-\$), a strong oxidant. [Method] Rat liver mitochondria were incubated with ONOO$^-\$ and mtMGST1 activity was measured with 5mM GSH and 1mM 1-chloro-2, 4-dinitrobenzene as substrates. MPT pore opening was evaluated with mitochondrial swelling which was detected as a change of absorbance at 540nm. [Results and Discussion] When mitochondria were incubated with various concentrations of ONOO$^-\$, mtMGST1activity was increased dose dependently and reached to 200% of control at more than 200µM. MtMGST1 activation by ONOO$^-\$ was inhibited by both MPT inhibitors including cyclosporin A (CsA) and ADP, and GST inhibitors such as cibacron blue. Mitochondrial swelling was induced by ONOO$^-\$ and the swelling was prevented by MPT- and GST- inhibitors, and by a reducing agent, dithiothreitol (DTT). A high molecular weight of mtMGST1 (dimer, oligomer) were detected in ONOO$^-\$-treated mitochondria in which MTP-and GST-inhibitors prevented the oligomerization of mtMGST1. These results show that mtMGST1 is oxidatively modified by ONOO$^-\$ resulting in its activation, which leads to the MPT pore opening, suggesting that the mtMGST1 acts as a part of the MPT pore.