30PE-43

A BASIC STUDY ON MIGRATION TO TISSUE OF SITE PROBE DRUGS BY USING A CELL MEMBRANE PLATE

Jin Tokunaga1, Norito Takamura1, Kenji Ogata1, Hiroki Yoshida1, Toyotaka Nishio2, Keiichi Kawai2
1School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki, 882-8508, 2School of Health Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, 920-0942, Japan

We have previously developed a simple, pharmaceutical distribution diagnostic method, to estimate the drug-binding capacity and its factor of each binding site on the human serum albumin (HSA) or α1-acid glycoprotein (AGP) in serum. In this study, to estimate the properties of drugs to migrate to tissue from the drug-binding capacity of HSA, we evaluated the cell membrane permeability of probe drugs used in the pharmaceutical distribution diagnostic method. Porcine kidney-derived LLC-PK1 cells (cell membrane) were seeded in a Transwell cell membrane plate and cultured. The upper and lower chambers of the cell membrane plate were assumed to be the tissue and vascular sides, respectively. Phenytoin (PHT) at a final concentration of 100 μM and valproic acid (VPA) at final concentrations of 100 and 200 μM were used as site I and II probe drugs, respectively. HSA at a final concentration of 0, 100, or 600 μM, bucolome (Buc) as a site I inhibitor at a final concentration of 100 or 600 μM, and oleic acid (Ole) as a site II inhibitor at a final concentration of 200 or 1,200 μM were added. In the presence of 100 or 600 μM HSA, 100 or 600 μM Buc caused a significant increase in PHT migration to tissue. Similarly, 200 or 1,200 μM Ole significantly increased VPA migration to tissue. We speculate that Ole inhibited the site for FFA on HSA and then site II. Ole is considered to inhibit the FFA site and site II and then to induce an allosteric change in site I, and, thereby, to increase protein binding; however, in the presence of 600 μM HSA, 1,200 μM Ole did not alter PHT migration to tissue. Further detailed studies on the migration of probe drugs to tissue are needed to estimate such drug migrations based on various clinical laboratory values, such as HSA, AGP, and FFA.

30PE-44

ENZYMATICAL SUPEROXIDE RADICAL GENERATION FROM FLUOROQUINOLONES

Masatake Kitamado1, Yousuke Muramoto1, Yoshihiro Mitani1, Tomohiro Karakawa1, Kazuko Nakagawa1 and Keizo Sato1
1Division of Pharmacology and Therapeutics, Graduate School of Medical and Pharmaceutical Sciences, and Center for Clinical Pharmaceutical Sciences, Kumamoto University, Kumamoto University, 5-1 Oe-Honmachi, Kumamoto, 862-0973, Japan

Fluoroquinolones, antimicrobial agents, are widely used in various clinical fields, such as respiratory-tract and urinary-tract infections, as the broad spectrum antimicrobial agents with excellent bioavailability. However, clinical experiences have shown the possible incidences of undesirable adverse effects including gastrointestinal, skin, hepatic, and central nervous system functions, and phototoxicity. While the UV-induced production of reactive oxygen species (ROS) plays a role in phototoxicity, the relationship between other adverse effects and ROS is still unclear. Even though free radical formation during the metabolism of fluoroquinolones in hepatic microsomes is investigated, a class of enzymes involved, species of ROS produced, and properties of radical production among fluoroquinolones are still unknown. Therefore, in this study, we evaluate the free radical production with enzymatic system. As results, highly purified cytochrome P-450 reductase (EC 1.6.2.4.) and NADPH were found to generate superoxide radical (O2-) from 3 different fluoroquinolones as identified by electron spin resonance spectroscopy (ESR) using the spin trapping method with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). These results indicate that fluoroquinolones can serve as a one-electron donor for superoxide generation by mediating enzymatical reaction of cytochrome P-450 reductase. The radical generation was highest with levofloxacin and lowest with gatifloxacin. In conclusion, because of the known the presence of cytochrome P-450 reductase in the cell at significant amount, the present findings might be an important implication for the plenty of free radical generation in various organs in addition to skin.