TRANSFORMATION OF POORLY WATER SOLUBLE DRUGS INTO IONIC LIQUIDS USING BIOCOMPATIBLE CATIONS: SOLUBILITY, PERMEATION AND CYTOTOXICITY STUDIES

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（生体適合性カチオンを用いた難水溶性薬物のイオン液体への変換：溶解度、透過性および細胞毒性研究）

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論 文 内 容 の 要 旨

The pharmaceutical industry is facing unparalleled challenges to develop the effective drug delivery systems for achieving targeted pharmacological response of many drugs because of their polymorphism, limited solubility, permeability and bioavailability. To address these limitations, an IL based formulation of APIs is a promising approach to design the smart delivery of drugs. Recently, ILs have been extensively used to convert the crystalline drugs into API-IL form because they provide improved aqueous solubility, enhanced absorption, desired dissolution rate and even targeting ability. API-ILs also would solve the problem of polymorphism, a significant challenge in drug delivery. In this thesis, we mainly focused on the synthesis and characterization of IL-forming cations as well as API-ILs and evaluated their feasibility for drug delivery systems.

In the **second chapter**, we concentrated on the synthesis and characterization of biocompatible amino acid esters (AAEs) based cations for transforming the crystalline salicylic acid (Sal) into liquid forms. A series of amino acids such as proline alanine, aspartic acid, glutamic acid, leucine, methionine, tyrosine and phenylalanine were selected to convert AAE cations by considering their reportedly low toxicities and physico-chemical activities. To investigate the biological activity of AAE cations in mammalian cell lines (L929 and HeLa), we synthesized proline-based AAE cations with various alkyl chain lengths (C_1–C_4) and found that the cytotoxicity of AAE cations mainly depends on the alkyl chain length in the ester group. The AAE cations toxicity increased with increasing the alkyl chain length from C_2 to C_4 and ethyl ester-based AAE cation showed comparatively lower toxicity among the other alkyl chain lengths. All synthesized amino acid ethyl esters (AAEt) cations were clear, colorless room temperature liquids and miscible in water except for L-tyrosine ethyl ester, with high purities (> 97.0%). The AAEt cations were characterized using ^{1}H and ^{13}C NMR, FTIR, elemental, and thermogravimetric analyses. However, the toxicities of the AAEt cations greatly increased with

inclusion of long alkyl chains, sulfur, and aromatic rings in the side groups of the cations. Among the synthesized AAEt's, AlaEt, AspEt, and ProEt had low cytotoxicities and were investigated as potential components for synthesis of salicylate ionic liquids (Sal-ILs) with the anionic Sal drug. All synthesized Sal-ILs were fully ionized and had high thermal stabilities. In contrast to free Sal, Sa-ILs were miscible with water at any ratio. The cytotoxicities of the Sal-ILs drastically increased compared with the AAEt's on incorporation of Sal into the cations, and were comparable to that of free Sal. In view of transdermal application, skin permeation studies were performed using a female Yucatan micro pig skin with temperature controlled Franz diffusion cell and demonstrated that the Sal-ILs ([Sal][AspEt]) penetrated through skin approximately nine times faster than that of the Sal sodium salt.

In the **third chapter**, we have dedicated our attention for evaluating the effect of cations in API-ILs on *in vitro* solubility and antitumor activity, and then *In vivo* biocompatibility, pharmacokinetics and antitumor efficacy studies. For these purpose, we selected several types of IL-forming cations from amino acids, cholinium, imidazolium, ammonium, phosphonium groups by considering their reportedly lower toxicities among their corresponding groups and were synthesized for converting the poorly water-soluble methotrexate into IL forms (MTX-ILs). All the synthesized MTX-ILs were solids except [Cho][MTX], [EMI][MTX] and [TBP][MTX], with high purities (> 97.0%). The synthesized MTX-ILs were characterized through ^{1}H NMR, FTIR, p-XRD, DSC and thermogravimetric analysis. The stoichiometry ratio and the fully ionization of MTX-ILs between MTX and the cations were clearly assessed by using ^{1}H NMR and FTIR, respectively, and confirmed by comparing with fully ionized sodium salt of MTX. The p-XRD spectra of MTX-ILs were confirmed the amorphous phase of the MTX-ILs while the free MTX was crystalline in nature. In thermophysical study, the MTX-ILs showed almost similar thermal stabilities than that of the free hydrate MTX. The DSC thermogram of free MTX exhibited a characteristic sharp endothermic peak at 154 °C, whereas the characteristic sharp endothermic peak of MTX-ILs were shifted lower from 154 °C, indicating a significant changes in their physical state from crystalline to amorphous. The solubility of the MTX-ILs was evaluated in both water and simulated body fluids (phosphate-buffered saline, simulated gastric, and simulated intestinal fluids). The MTX-ILs showed aqueous solubility at least 5000 times higher than that of free MTX and two orders of magnitude higher compared with that of a sodium salt of MTX in both water and simulated body fluids. An assessment of the *in vitro* antitumor activity of the MTX-ILs in a mammalian cell line (HeLa cells) was used to evaluate their cytotoxicity. The amino acid ethyl ester-MTX IL ([ProEt][MTX] and [AspEt][MTX]) showed similar solubility as the MTX sodium salt but it provided improved *in vitro* antitumor activity. In view of practical application, in vivo

biocompatibility, pharmacokinetics and antitumor efficacy of methotrexate ionic liquid moieties were investigated for envisaging their therapeutic application as a biocompatible component of a drug delivery system. In pharmacokinetic study through oral admiration, the MTX-ILs showed significantly higher pharmacokinetic parameters than that of sodium salt of MTX especially amino acid ethyl esters based ILs (AAEt-MTX). The bioavailability of AAEt-MTXs showed at least six times higher than that of sodium MTX. Even though, AAEt-MTXs also orally biocompatible in mutli-doses of every after days at 15 mg/kg. The antitumor efficacy of MTX-ILs and sodium MTX are presently under investigation.

In the **fourth chapter**, we focused our attention for introducing a potent biocompatible cation to ionic liquefied the poorly water insoluble crystalline drugs and envisaging their biomedical applications. We chose the N-methyl-2-pyrrolidone (NMP) because of the advantageous properties such as biodegradability, the Food and Drug Administration (FDA)-approved enlisted as generally recognized as a safe solvent with low toxicity and well-known skin enhancer for topically applied drugs. The synthesized NMP cation was liquid at room temperature and showed lower cytotoxicity than that of conventional counter cations in mammalian cell lines (NIH3T3 and L929 cells). The synthesized NMP-based ionic liquid (NMP-IL) was characterized using ^{1}H & ^{13}C-NMR, FT-IR, DSC and TGA. In view of topical application, skin permeation studies were performed using a female Yucatan micro pig skin with temperature controlled Franz diffusion cell and demonstrated that NMP-IL showed enhanced skin penetration, and enriched drug accumulation 2.6 times higher than that of IL [Cho][Ibu] in the target tissue.

In conclusion, the obtained results (**chapter 2, 3 & 4**) suggest that AAEs or NMP could be a potent biocompatible counter ion to eliminate the use of traditional toxic solvents for oral/ topical/ transdermal delivery of poorly water-soluble drugs, since they allow an improved solubility and bioavailability or permeability of the studied APIs.