Summary of Doctoral Dissertation

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Enhancing Intracellular Delivery and Pharmacokinetics of Small and Hydrophobic Drugs Using Nanoparticles

(疎水性低分子の体内動態・細胞内動態を制御する新規ナノ粒子法)

Recent advances in biotechnology made the production of macromolecular drugs, such as antibodies and nucleic acids, much easier and promising for the treatment of incurable diseases. While many scientists think that the era of small molecular weight drugs (small drugs) has been replaced by macromolecules, >50% of the innovative drug approvals in the last 3 years consist of small drugs (source: FDA center of drug evaluation and research). This indicates that small drugs still have big presence in the drug discovery and development process. The fact that most of these drugs are hydrophobic in nature makes their administration problematic. Thus, it is of substantial importance to provide pharmaceutical solutions to enhance the performance of small hydrophobic drugs and keep their flow in the innovative drug pipelines. In this study, formulation of small hydrophobic drugs in nanoparticles (NPs) has been employed to control their solubility, intracellular delivery and pharmacokinetics.

Curcumin, a small hydrophobic compound of the spice turmeric, has strong anti-cancer and anti-inflammatory effects and a high margin of safety. However, the clinical translation of curcumin is old yet unresolved problem. This arises from the poor physicochemical properties of curcumin such as low aqueous solubility and rapid degradation. In this study, we adapted a bottom-up design approach for the formulation of curcumin-loaded NPs. From a preliminary screening of the solubility of curcumin in different materials, the nonionic surfactant Tween 80 showed a substantially high affinity to curcumin. Tween 80 was used to construct 2 structurally-distinct NPs: a core-shell and a vesicular type NPs (namely the Nanoemuslion, NE and the Niosome, NIO, respectively). Surprisingly, although these 2 NPs have comparable particle sizes, the NIO showed significantly potent *in-vitro* anticancer effect and higher intracellular accumulation of curcumin compared to the NE. This unexpected difference between the NE and the NIO promoted us to conduct mechanistic comparison and clarify their mechanisms of drug delivery.

It is generally known that drug-loaded NPs exert their action either by releasing the drug in the extracellular region followed by entry of the drug in the free form, or by whole particle endocytosis prior to drug release inside the endosome/lysosome. Using fluorescence activated cell sorting (FACS) and confocal microscopy, we found that endocytosis has negligible effect on the overall intracellular delivery of curcumin, which could be attributed to the possible deactivation of curcumin in the lysosome. Further, the release rate of curcumin was evaluated using the dialysis method. Both the NE and the NIO show slow drug release in aqueous conditions, which could explain the weak anti-cancer effect of the NE but not the NIO. Therefore, it became evident that the NIO delivers curcumin to the cytosol *via* unknown non-endocytic pathways. We hypothesized that curcumin is directly delivered into the cytosol *via* a cell membrane-mediated transfer when introduced in the NIO form. In other words, curcumin is being transferred from the NIO membrane to the cell membrane upon collision and contact. The kinetics of curcumin transfer upon mixing with cell membrane-mimicking species, i.e. liposomes, were evaluated using Förster Resonance Energy Transfer (FRET) between curcumin and an acceptor probe. It was found that curcumin transfers very efficiently when formulated in the NIO, but not the NE. We

also investigated the critical factors that dictate the membrane-mediated transfer efficiency of curcumin from the NP, and found the presence of a hydrophobic core reduces it. To the best of our knowledge, this was the first report establishing a link between the structure of a NP and its efficiency in drug transfer.

The NE, for it is easier to prepare and load curcumin high concentration and showed slower transfer of curcumin *in-vitro*, was chosen for the *in-vivo* experiments. Soon after intravenous injection of curcumin-NE, curcumin showed rapid release and elimination from the blood plasma, although the NE particles exhibited long circulation. This phenomenon is known by premature drug release and comprises one of the major causes of therapeutic failure of small hydrophobic drug-loaded NPs. We identified 2 major factors that play an important role in the control of plasma retention of curcumin in mice: Drug Loading (DL), which is a measure of the weight of drug relative to total weight of the NP, and drug-carrier interaction affinity in the NP core.

The DL was found inversely dependent on the curcumin plasma retention, i.e. NPs with low DL are better in prolonging the circulation time of curcumin. Next, DL was fixed and the composition of the NE was changed. By changing the surfactant from Tween 80 to Pluronic F127, the plasma retention of curcumin increased as this modification increases the hydrogen bond interaction between curcumin and the methoxy groups of the new surfactant. The plasma retention of curcumin was further improved by adding a novel oil-like material in the NE core, which offers curcumin an additional type of non-covalent interaction. This material was synthesized in our laboratory and showed significant enhancement in curcumin solubility.

Combined together, a reduced DL ratio (from 1% to 0.5%) and introduction of multiple non-covalent interactions between curcumin and the NE core resulted in a 12.4 folds increase in the area under the curve (AUC) of the curcumin plasma profiles. For moderately hydrophobic molecules such as curcumin, covalent modification of the drug to increase its molecular weight and hydrophobicity is one of the gold standards to reduce its rate of release. However, this approach requires the cleavage of the prodrug and release of the active moiety for a pharmacological effect to happen. Furthermore, for many natural drugs like curcumin, which gain a Generally Recognized As Safe (GRAS) status from the FDA, any minor modification in their chemical structures will make them lose the status. Herein we report on enhancing the plasma retention of curcumin using non-covalent modification, which is a promising strategy for maximizing the therapeutic effect of the unmodified form of the drug.

Finally, the usefulness of the newly synthesized oil-like material as a general solvent for small hydrophobic molecules was investigated. The saturated solubility of quercetin, paclitaxel and silicon phthalocyanine in the oil-like material was measured and compared to that in coconut oil as a model triglyceride oil. Interestingly, these molecules showed substantially high saturated solubility values when dissolved in the novel oil-like material. Moreover, paclitaxel-loaded NE composed of the oil-like material was intravenously injected and the AUC was improved 4.2 compared to coconut oil core, confirming the universality of the novel material as a solvent and vehicle for various small hydrophobic compounds.