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The study investigated the aerosolization and inhalation profiles of rifampicin-oleic acid first generation liquid and solid nanoemulsions and their respective chitosan- and chitosan-folate conjugate-decorated second and third generation nanoemulsions. The liquid nanoemulsions were prepared by spontaneous emulsification method and had their size, zeta potential, polydispersity index, morphology, pH, viscosity, surface tension, density, refractive index, drug content, drug release, aerosolization and inhalation profiles characterized. The first, second and third generation nanoemulsions had average droplet sizes of 43.89 ± 0.36 nm, 52.12 ± 0.36 nm and 59.69 ± 0.26 nm, with narrow polydispersity indices at 0.16 ± 0.03, 0.25 ± 0.03 and 0.23 ± 0.01 respectively. They exhibited desirable pH, surface tension, viscosity, refractive index, density, and viscosity attributes for pulmonary rifampicin administration. The second generation nanoemulsion was characterized by relatively low levels of burst drug release due to intimate chitosan packing at the oil globules’ surfaces and viscosifying effect on continuous phase, which was unattainable by the branched folate conjugate of chitosan. All nanoemulsions demonstrated more than 95% aerosol output and inhalation efficiency greater than 75% when delivered by nebulization. The aerosol output, aerosolized and inhaled fine particle fractions were primarily governed by the size and surface tension of nanoemulsions in an inverse relationship. The first, second and third generation nanoemulsions were converted to their corresponding solid counterparts by spray drying method. The spray-dried solid first, second and third generation nanoemulsions achieved particle sizes of 7.05 ± 0.38 μm, 7.96 ± 0.33 and 5.45 ± 0.38 μm respectively, with sustained drug release behavior as compared to their associated nanoemulsions due to their large particle sizes and solid nature. The powder exhibited an aerosol output of > 65% when delivered using Handihaler. The mass median aerodynamic diameters of < 5 μm was achieved for all spray-dried solid nanoemulsions, due to their lower tapped densities resulting in inhaled fraction of > 30%. Among physicochemical properties of spray-dried nanoemulsions, increased circularity and lower tapped density have been found to improve aerosolization of powder from dry powder inhaler, while higher span value tends to improve the FPF. Due to significantly higher aerosolization potential and inhalation efficiency of liquid nanoemulsions, they were evaluated for their cellular internalization, safety and pharmacokinetics behaviors in cell culture and animal models. A significantly higher level of cellular internalization was observed with third generation nanoemulsion when compared to second generation liquid nanoemulsion due to double receptors targeting in the former via folate and acetylglycosamine moiety of chitosan. The liquid nanoemulsions were regarded as safe and biocompatible with reference to rifampicin in therapeutic doses, because macrophages remain viable (> 80%) following their incubation with nanoemulsions. The pharmacokinetics analysis revealed that nanoemulsion succeed in maintaining therapeutic level of drug in the plasma for 16 h after intratracheal drug administration, with higher lung drug concentration in case of third generation nanoemulsion. Thus, both liquid and solid nanoemulsions are suitable for use as rifampicin carrier in the treatment of tuberculosis.

This project focused on formulation and investigation of interplay effects of ethosomes, ethanol and microwave for enhanced skin drug retention with minimal systemic absorption. The microwave was used to modify the skin barrier properties to enhance ethosomes and/or drug penetration/and drug retention which is detrimental to treat local malignant melanoma and to enhance patient compliance. Ethosomes are known to fuse with skin to enable local drug retention. Pre-treatment of skin with microwave and applying liquified medicine is deemed to “cement” the skin thereby raising skin drug deposition. 5-fluorouracil loaded ethosomes were prepared and subjected physicochemical characterization. The molecular characteristics of untreated, microwave and/or ethosomes and/or ethanol-treated skins were examined by ATR-FTIR and raman spectroscopy, DSC and SEM techniques. The skin drug retention was promoted using larger ethosomes with negative zeta potentials that repelled anionic lipids of skin and hindered vesicle and/or anionic drug penetration into deep layers. Due to low ethanol, which were less able to fluidize the lipid and defluidize the protein domains at epidermis to enlarge aqueous pores for drug permeation. Pre-treatment of skin by 2450 MHz microwave for 2.5 min further increased skin drug penetration and retention of E5 ethosomes and provided lower drug permeation than cases treated for 1.15 min and 5 min. Pre-treatment skin with microwave fluidized lipid and defluidized protein domains of skin that promoted transdermal drug penetration. A 2.5 min treatment however might be accompanied by specific dermal protein fluidization via C=O moiety which translated to macromolecular swelling, narrowing of intercellular spaces at lower skin layers, increased drug retention and reduced drug permeation. Ethosomes in combination with microwave at 2450 MHz for 2.5 min promoted significant drug deposition in skin from ethosomes in vivo with reduced systemic absorption. Pre-treatment of human melanoma cells with microwave exerted cytotoxic effect and also facilitated the intracellular ethosomes accumulation by fluidizing the cell membrane phospholipids reflected by a significant increase in wavenumber corresponding to symmetric phosphate moiety. The endocytosis was primarily promoted by lipid rafts pathway where a significant reduction in fluorescence intensity was observed when melanoma cells were pre-incubated with nystatin. Combined microwave and ethanol pretreatment of skin increased skin drug retention and decreased permeation of aqueous 5-fluorouracil solution. The combination fluidized the skin lipidic domains, defluidized the hydrophilic regimens causing an increase in aqueous pores population and their size. The summative effect translated into an increased drug penetration, permeation and retention of drug solution in the skin. When microwave pre-treatment was combined with 100 μl ethanol, rapid movement of ethanol from epidermis to dermis under the gravity bringing extracted epidermal lipids downwards and accumulating them in dermis in addition to fluidization of the extracellular proteins at C-N moieties. The expanded proteins structure and epidermal lipids accumulation in dermis promoted skin drug retention by narrowing the permeation pathways and formation of an additional lipid barrier consisting of ceramide and palmitic acid in dermis.