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Research Article

Rational Design and Characterization of a Nanosuspension for Intraoral Administration Considering Physiological Conditions

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ABSTRACT

The oral cavity displays an attractive route in drug administration that is not associated with gastric transit and hepatic first-pass metabolism. However, limiting factors for an efficient transit of drugs through the oral mucosa are poor water solubility and permeability. Hence, various strategies exist to enhance solubility. Specifically, nanotechnology has attracted much research interest in the past decade. This study aimed at developing a stable nanosuspension of the model compound phenytoin via wet media milling. The nanosuspensions were carefully characterized regarding hydrodynamic particle sizes, crystallinity, and dissolution characteristics under nonphysiological or physiological (salivary) conditions. The permeability of bulk phenytoin and nanophenytoin through a buccal *in vitro* and *ex vivo* model was investigated, and the apparent permeability coefficients were determined. Moreover, cytotoxicity studies were conducted. The addition of Tween 80 as stabilizer resulted in a stable crystalline nanosuspension (330 nm). The solubility characteristics significantly increased under salivary conditions, which further impacted the permeability, as the steady state appearance rate of nanosized phenytoin was 1.4-fold higher. Cytotoxicity studies demonstrated that bulk-/nano-phenytoin exhibited no harmful effects. It can be concluded that the salivary environment (i.e., ionic strength, pH) strongly impacts the solubility and consequently the permeability of crystalline nanosuspensions across the buccal mucosa.

Introduction

Oral administration of drugs with intestinal absorption is the traditionally preferred route of drug delivery. However, it faces serious obstacles including pH changes, enzymatic degradation of the active pharmaceutical ingredient (API), and hepatic first-pass metabolism, making this route a considerable challenge for molecules.[1-3] Hence, alternative sites for drug administration, which avoid these obstacles, are increasingly sought.

The oral cavity displays an attractive route for drug administration that is not associated with this phenomenon. However, it is equipped with distinct protective barriers, such as saliva, which acts as lubricant but also is likely to impact the solubility and permeability behavior of certain drug candidates. The lining mucosa (i.e., buccal, sublingual) represents the largest surface area in the oral cavity and comprises a nonkeratinized stratified squamous epithelium, which consists of 40-50 cell layers with a thickness of approximately 500-600 μm.[4] It is formed from 4 different morphological layers, namely the basal layer, the prickle cell layer, the intermediate layer, and the superficial layer. Once cells leave the basal layer, they enter differentiation and become large and flat. Moreover, the prickle cells contain membrane-coating granules. These cytosolic granules fuse with the cell membrane and extrude lipids into the intercellular spaces. Thereby, they constitute a strong barrier and limit the penetration of drugs specifically in the top third region of the epithelium.[3,5] Apart from anatomic and physiological barriers, permeation of substances is also impacted by the drug's physicochemical properties (i.e., solubility, ionization, lipophilicity, molecular weight).[3,4] The majority of drugs are transported passively through the buccal mucosa,[3] either via the paracellular (i.e., in between epithelial cells) or via the transcellular route (i.e., through epithelial cells).[6-9] The former pathway is predominantly available for hydrophilic molecules having small molecular sizes.

The latter one is the preferred route for lipophilic drugs, which interact with the lipophilic cell membrane.[2,10,11]

However, because of the increasing number of drugs that show poor solubility and/or permeability, diffusion through oral mucosae is often hindered. Hence, different strategies have been used to enhance

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drug transport through buccal tissues. These strategies include permeation enhancers and vehicles and cosolvents.[2,10,11] Permeation enhancers, such as bile salts, surfactants, and fatty acids, are applied to change the mucus rheology, increase the fluidity of the lipid bilayer membrane, solubilize the intercellular lipids (to facilitate paracellular transport), or increase the flux of the drug by increasing the thermodynamic activity.[2,12-15] However, the exact mechanism is often not well understood, and knowledge of possible adverse effects of penetration enhancers on biological tissues is lacking.[16]

Another alternative for overcoming insufficient transport across buccal tissue is to enhance the solubility of poorly soluble drugs. Particle size reduction to the nanoscale is a promising strategy and can be achieved either by top-down approaches (i.e., breakage of large particles into nanocrystals) or by bottom-up approaches (e.g., nanoparticles are built up from molecules via precipitation).[17-23] The former one results in nanosuspensions, which are carrier-free-submicron colloidal dispersions of drug particles in an aqueous medium, stabilized by polymers and/or surfactants. These stabilizing agents are used to prevent agglomeration, caused by the higher surface energy of the nanomaterial.[21,24-29] Moreover, sedimentation and/or crystal growth might occur[30,31]; thus, for applicability in the oral cavity, nanosuspensions need to be further processed into solid orodispersible formulations, including oral lyophilisates, orodispersible tablets, orodispersible granules, and orodispersible films.[32] This facilitates easier handling, shipping, and storage of the delivery form and improves efficacy, safety, and stability.[26,33,34] Most commonly, freeze drying, spray drying, and vacuum drying are conducted to result in a nanopowder, which can be further incorporated into tablets, granules, and films.[35-37] More importantly, nanosuspensions can be transferred into the desired dosage directly from the liquid phase using casting methods,[38-40] printing technologies,[41,42] or the recently developed continuous nanoextrusion process.[43,44]

Although it is known that nanoformulations can improve drug delivery, the understanding of how they interact with the anatomic and physiological barriers in the oral cavity is lacking. In this study, we aimed at investigating whether the salivary environment, such as ionic strength and pH, impacts the solubility behavior of a nanosuspension and as a further consequence the permeability of the drug across the buccal mucosa. To this end, phenytoin (5,5-diphenylhydantoin), which is an antiepileptic and antiarrhythmic drug[45] showing poor solubility (11.5 ± 0.5 μg/mL[44]) and a slow rate of absorption after oral intestinal administration,[12,46,47] was used as model drug. A stable aqueous nanosuspension of the model compound was prepared via 1-step wet media milling. Various stabilizers were tested, and the nanosuspensions were carefully characterized regarding hydrodynamic particle sizes, crystallinity, and dissolution characteristics. The most stable and promising nanosuspension was identified, and the particle behavior in terms of solubility was recorded under salivary conditions (i.e., mimicking the pH and the ionic strength). Moreover, permeability studies were conducted. For this, human buccal TR 146 cells were used and cultured on Transwells to evaluate the transport mechanism involved. Additionally, permeability studies of the bulk suspensions and nanosuspensions were conducted using a standardized porcine *ex vivo* model.[48-50] Because it is known that by reducing the particle size from the microscale to the nanoscale, the specific nanomaterial changes its properties, which might result in adverse effects,[34,49,51-59] cytotoxicity studies were conducted as safety feature.

Materials and Methods

Materials

Phenytoin (5,5-diphenylhydantoin) from Sigma–Aldrich (Munich, Germany) was used as model API. The stabilizers Tween 20 (P20), Tween 80 (P80), and methanol (CHROMASOLV®, for high-performance liquid chromatography [HPLC], ≥99.9%) were purchased from Simga–Aldrich. Kolliphor EL (KEL), Kolliphor RH40 (KRH40), Kolliphor P188 (KP188), and Kolliphor P407 (KP407) were donated by the manufacturer BASF (BASF SE, Ludwigshafen, Germany). Ultrapurified water (i.e., Milli-Q-water [MQ-water], Millipore S.A.S., Molsheim, France) was used for all experiments. For cell culture tests, Dulbecco's Modified Eagle's medium (DMEM; Gibco, Life Technologies Corporation, Paisley, UK), DMEM with 10% of fetal bovine serum (FBS; Sigma–Aldrich), and Hank's buffered salt solution (HBSS; Gibco, Life Technologies Corporation) were used. For *ex vivo* studies, PBS (Gibco, Life Technologies Corporation) and Krebs Buffer (Krebs–Ringer Bicarbonate Buffer with 1.8 mg/L glucose, without $CaCl_2$ and $NaHCO_3$; Sigma–Aldrich) were applied.

Methods

Nanosuspension Preparation

Contact Angle. Contact angle measurements were conducted using the sessile drop method (Easy Drop; Krüss, Hamburg, Germany) to obtain preliminary indications of the most appropriate stabilizer for wetting the newly generated drug surface. For this, phenytoin powder compacts (500 mg) were prepared. A drop of 10 μL of water and stabilizer solutions (5% [w/w] stabilizer diluted with MQ-water) was dispensed onto the sample surface, and images were captured by camera (Stingray F046B; Allied Vision Technologies). The contact angle was calculated by the instrument (DSA1 "drop shape analysis," Krüss) by fitting a mathematical expression to the shape of the drop and then calculating the slope of the tangent to the drop at the liquid-solid-vapor interface line. The experiments were carried out in triplicate, and average values and standard deviations were calculated. All measurements were performed in air under ambient conditions.

Surface Tension of the Stabilizer Solutions. For determining the surface tension of the various stabilizer solutions, the Easy Drop System (Krüss) was used. The surface tension was calculated using the L-Y method considering the densities of stabilizer solutions. The solution densities were determined via sound velocity measurements using a DSA 5000M (Anton Paar GmbH, Graz, Austria) at 20°C.

Wet Media Milling. Phenytoin nanocrystals were prepared by wet media milling using various stabilizers (i.e., P80, P20, KEL, KRH 40, KP188, KP407) as described previously by Baumgartner et al.[44] After diluting 10 g of the stabilizer with 200 mL MQ-water, 40 g phenytoin was dispersed in this aqueous stabilizer solution. Yttrium-stabilized zirconium beads (600 g, 0.5 mm in diameter) were used as a milling agent. Milling was performed with a planetary ball mill (Retsch PM 100; Retsch GmbH, Haan, Germany) equipped with a zirconium oxide grinding bowl (500 mL) at 250 rpm for 24 h. The experiments were carried out at ambient temperature. After milling, the grinding beads were separated from the nanocrystals by sieving.

Nanosuspension Characterization

Particle Size and Zeta Potential Analyses. The prepared nanosuspensions were investigated by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) equipped with a 532-nm laser. PCS yields the mean diameter as a light intensity weighted size of the bulk population (z-average) and the polydispersity index (PdI) as a measure for the width of the particle size distribution.[60] To prevent nanoparticle dissolution during measurements, nanosuspensions were diluted in a saturated phenytoin–water solution.[61,62] The measurements were