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Curcumin Cocrystal Micelles—Multifunctional Nanocomposites for Management of Neurodegenerative Ailments

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ABSTRACT

Curcumin, a potent antioxidant polyphenol with neuroprotective and antiamyloid activities, has significant potential in the treatment of neurodegenerative disorders such as Alzheimer's disease. However, its clinical translation is delayed due to poor bioavailability. For effective use of curcumin in Alzheimer's disease, it is imperative to increase its bioavailability with enhanced delivery at a therapeutic site that is, brain. With this objective, pharmaceutical cocrystals of curcumin were developed and incorporated in micellar nanocarriers for nose-to-brain delivery. For cocrystals, an antioxidant hydrophilic coformer was strategically selected using molecular modeling approach. The cocrystals were formulated using a planetary ball mill, and the process was optimized using 3^2 factorial design followed by characterization using differential scanning calorimetry, X-ray diffraction, and Fourier-transform infrared spectroscopy analysis. The cocrystal micelles exhibited globule size of 28.79 ± 0.86 nm. Further, curcumin cocrystal and co-crystal micelles exhibited a significantly low (p value <0.01) IC_{50} concentration for antioxidant activity as compared to curcumin corroborating superior antioxidant performance. *In vivo* studies revealed about 1.7-fold absolute bioavailability of curcumin cocrystal micelles with C_{max} of 1218.38 ± 58.11 ng/mL and showed significantly high brain distribution even beyond 6 hours of dosing. Thus, the studies confirmed enhanced bioavailability, higher brain uptake, retention, and delayed clearance with curcumin cocrystal micellar nanocarriers.

Introduction

Oxidative stress is reported to be a hallmark of neurodegenerative disorders including Alzheimer's disease (AD). The oxidative stress is triggered due to increased oxidation of lipids, proteins, nucleic acids, and so forth that results in the formation of reactive oxygen species leading to neuronal damage. Furthermore, the neurodegeneration and associated dementia is augmented as neuronal capacity to compensate for this stress is compromised with aging.[1,2] In this context, polyphenols have been reported to exhibit strong antioxidant and anti-inflammatory properties which could be beneficial in oxidative stress management. As a result, their use as supportive therapy and dietary food supplement is widely suggested in the recent years.[3]

Amongst these, curcumin, a potent antioxidant polyphenol extracted from rhizomes of turmeric (*Curcuma longa* L)—a well known curry spice in India and native countries—is on forefront as it exhibits additional pharmacological activities beneficial in AD management, and thus, its use is strongly recommended. Briefly, curcumin is a strong antioxidant that not only inhibits but also scavenges free radicals, reduces lipid peroxidation, and increases superoxide dismutase, Na^+-K^+ ATPase activity to compensate cellular oxidative stress.[4-7] Its cytoprotective activity is also attributed to upregulation of hemoxygenase that nullifies cellular oxidative stress via native glutathione antioxidant pathway. Its anti-inflammatory potential is considered synonymous to that of nonsteroidal anti-inflammatory agents, and it is reported to inhibit cyclooxygenase, phospholipase, and chemotaxis of monocytes (by inhibiting Aβ-induced expression of Egr-1 protein expression that accelerated monocyte chemotaxis) that arrest neuronal inflammation.[4] It is also reported to downregulate intracellular cytokines viz. IL-12 p40/p70 and IL-12 p70 expression and thereby offers cellular protection.[4] Curcumin is also reported as a strong chelator of redox-active metal ions such as iron and copper and thus can arrest metal-induced induction of NF-kappa that causes inflammation and AD progression.[4] Promisingly, curcumin is also reported to inhibit amyloid ß (Aß) plaque aggregation and destabilize the Aß fragment. This is proven with *in vitro* experiments that showed destabilization of Aß plaque followed by an *in vivo* study

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that revealed reduction in senile plaque density upon 7-day curcumin treatment in mice model.[4-6] Another study on mice model exhibited 40% reduction in Aß levels of animals treated with curcumin than the untreated group.[4,7]

In addition, a survey has shown 4.4 times less prevalence of AD in Indian population between the age of 70 and 79 years than that of the US population and was correlated to the use of curry spice in India.[8] Another survey indicated that population (age between 60 and 93 years) who ate curry containing curry spice occasionally (less than once a month) and often (more than once a month) expressed better cognitive function than those who never consumed curry.[9] These results have been correlated to the potential of curcumin as an AD therapeutic active. Furthermore, Ayurveda, Siddha, and Unani (traditional Indian medicinal systems) also report medicinal uses of curcumin such as pain relieving, anti-inflammatory, antioxidant agent, and is being defined as "cleanser of body."

Despite of such a potent activity, clinical performance of curcumin is highly compromised due to extremely poor oral bioavailability (22-41 ng/mL followed by a plateau over 4 weeks at the dose of 8 g/d in phase II clinical trials), very poor aqueous solubility (11 ng/mL in aqueous buffer pH 5.0), instability at neutral and basic pH, and very short half-life.[10,11] Literature reports a range of strategies to increase bioavailability of curcumin and other poorly bioavailable drugs, some of which include development of polymeric, lipid, micellar nanocarriers, hydrogels, curcumin-cyclodextrin complexes and so forth[11-15] For effective use of curcumin in AD therapeutics, it is not only necessary to increase its bioavailability but also imperative to enhance the curcumin delivery at a therapeutic site that is, brain. With this goal, this article reports the development of pharmaceutical cocrystal of curcumin and incorporation thereof in micellar nanocarrier system for facilitated delivery via nose-to-brain route.

Pharmaceutical cocrystals are defined as single-phase crystalline structures comprising a therapeutic active and a coformer (pharmacologically active/inert) in a specific stoichiometric ratio. These are identified as nonionic supramolecular crystal lattice that uniquely retain the pharmacological activity of the drug but allow alteration in physical properties owing to the coformer and newly engineered crystal lattice. Literature reports a wide range of pharmaceutical cocrystals to modulate physicochemical properties and clinical performance of drugs.[16,17] This concept can also be constructively proposed for poorly water-soluble drugs, wherein a development of solubility enhanced cocrystals can be achieved by using a hydrophilic coformer. In view of this, the research work, herein, investigated the development of curcumin cocrystals with a hydrophilic coformer. The novelty of this research work lies in selection of smart hydrophilic coformer with additional antioxidant activity. This strategic use of functional coformer was hypothesized to not only enhance the aqueous solubility curcumin but also to augment the antioxidant activity of the resulting crystal lattice which is a beneficial phenomenon in case of AD-associated oxidative stress management.

For the fabrication of cocrystals, literature reports a wide range of techniques that can be broadly classified in 2 categories viz. high shear techniques (e.g., cogrinding, kneading, and high shear mixing [ball mill and vibrating mill]) and solvent-based techniques (e.g., slow solvent evaporation, sublimation, antisolvent method, and supercritical solvent).[16] Herein, we have investigated a planetary ball mill—assisted high shear technique for development of cocrystals as an effective green process that avoids use of solvents and enables easy scale-up in a Quality by Design framework.

From enhanced brain delivery per se, intranasal route of administration is gaining wide attention in the recent years as it represents the shortest and the most accessible brain delivery route via olfactory region and thus was selected as the choicest delivery route for this investigation.

As the developed curcumin cocrystals were proposed for intranasal delivery, micellar nanocarriers of curcumin cocrystals were developed in view of the following reasons. First, cocrystals can be stabilized using micellar assemblies as they provide thermodynamic stability to the cocrystals, and this phenomenon is strongly established through previous research.[18-20] Second, micelles are also reported to be compatible and effective for intranasal delivery owing to their colloidal properties and nanometric size distribution.[21,22]

Briefly, the research describes systematic crystal engineering approach towards development of pharmaceutical cocrystals of curcumin using a functional coformer and incorporation thereof in micellar nanocarrier spray system for intranasal delivery to target brain. The developed formulation was thoroughly investigated for physiochemical properties followed by *in vivo* pharmacokinetic and brain biodistribution studies to corroborate the proposed brain tissue bioenhancement hypothesis.

Materials and Methods

Materials

Curcumin (95% w/w pure) was received as a gift sample from Konark Herbals Ltd. (Mumbai, Maharashtra, India). Coformer A, dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT), Dulbecco's Modified Eagle's Medium (DMEM), and 1,1-diphenyl-2-picryl hydrazyl (DPPH) were purchased from Sigma-Aldrich Inc. (Mumbai, Maharashtra, India). Tween 80 and PEG 400 were procured from S.D. Fine Chemical Ltd. (Mumbai, Maharashtra, India). Solutol HS 15 (BASF India Ltd., Mumbai, Maharashtra, India), TrypLE™ (Express Enzyme, Mumbai, Maharashtra, India), 96-well tissue culture plates (Corning Costar Inc.), dialysis membrane—50 KD (Himedia Inc., Mumbai, Maharashtra, India), 0.45 and 0.22 μm filtration membranes (Pall Life Sciences, Mumbai, Maharashtra, India), Equadel™ 100 (Valois India Ltd., Mumbai, Maharashtra, India) were also purchased. Unless otherwise indicated, all other chemicals used were of analytical grade, and freshly prepared double-distilled water was filtered through 0.22-μm membrane before use.

Development of Curcumin Cocrystal

Selection of Coformer

The specific class of "antioxidant hydrophilic coformers" was tested with a dual objective. First objective was to enhance the overall antioxidant potential of the curcumin cocrystal which will be beneficial in AD-associated oxidative stress management. Second objective was to increase the aqueous solubility of curcumin cocrystals as compared to curcumin alone. For the selection of coformer, a systematic approach was investigated that included screening of various physicochemical parameters followed by molecular simulation studies as described in the following section.

Hildebrand Solubility Parameter. Pharmaceutical cocrystals are nonionic supramolecular crystal lattice of drug and coformer that exhibit solid-state miscibility at molecular level allowing nonionic interactions. With this in view, a theoretical miscibility determination tool, Hildebrand solubility parameter (δ) was employed as a screening parameter, and "δ" value of curcumin and proposed list of coformers were calculated using Equation 1.[23,24]