NANOPARTICLES AS PLATFORMS FOR DELIVERY OF CURCUMIN

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Abstract. Curcumin, the active polyphenol isolated from Curcuma Longa, exhibited potent pleiotropic, antineoplastic activity, attributed with minimal toxicity to normal cells. Unfortunately, the clinical implementation of curcumin is limited due to its instability in physiological pH, low aqueous solubility (11 ng/ml) associated with extremely low systemic bioavailability after oral administration of 8 g/day. An intriguing approach to overcome these limitations is incorporation of curcumin in nanoparticles as delivery platforms such as solid lipid nanoparticles, nanoemulsions, liposomes and macrocyclic cavitands. A promising strategy for improvement of unfavorable physicochemical characteristics of curcumin consisting of its simultaneous loading in the phospholipid bilayer membrane and in the aqueous cavity of liposomes as inclusion complex with macrocyclic cavitands is presented.

Key words: curcumin, nanoparticles, liposomes, macrocyclic cavitands

Introduction

Curcumin is a biologically active substance, extracted from the rhizome of *Curcuma Longa* [1]. From ancient times, curcumin is used widely in East Asia, not only as a spice, but also as a medicine for treating liver and gall- bladder diseases, inflammatory and rheumatic conditions, anorexia, etc. [2]. The first isolated curcumin was generated in 1815, from the rhizome of *Curcuma longa L*. by Vogel and Pelletier, and in 1910 it was identified as dipheruloilmethane [1,7-Bis(3-methoxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione], classified in the group of polyphenol compounds [3]. A large number of contemporary pharmaceutical research studies showed that curcumin possesses powerful anti-inflammatory and antitumor effects, owing to the pleiotropic influence on the inflammatory cascade and the malign signal cell pathways [4]. Curcumin also possesses strong antioxidant activity in neutral and acid conditions. It influences and also modulates the activity of a number of enzymes [5] and genes, resulting in activation of signal cascades of the programed cell death in the tumor cells [1, 4, 6].

However, the main mechanism of pharmacological activity of curcumin is associated with the inhibition of nuclear factor κB (NFκB), connected with expression and regulation of various genes implicated in tumor biology of different malignances like colon cancer, leukemia, multiple myeloma etc.

Regretfully, the enormous therapeutic potential of curcumin can`t be exploited in clinical practice, due to its extremely unfavorable physicochemical and pharmacokinetic characteristics, and also due to the instability in systemic circulation [5]. Curcumin is characterized with extremely low solubility in water (11ng/ml) and significant presystemic biotransformation, mainly *via* glucuronide and sulfate conjugation [7]. Consequently, curcumin shows very low bioavailability after oral application, and impossibility to achieve therapeutic concentration in the target areas. In order to optimize the unfavorable pharmacokinetic and physicochemical characteristics, enormous effort is directed towards development of nanoscale systems as platforms for its delivery such as solid lipid nanoparticles, nanoemulsions, liposomes and macrocyclic cavitands [8].

Curcumin loaded solid-lipid nanoparticles

One of the approaches to improve the poor aqueous solubility and instability as well as to prolong the

cytotoxic activity of curcumin is the encapsulation into solid lipid nanoparticles (SLNs). Many research studies have shown that by this incorporation into lipid nanoparticles the permeability and bioavailability, of curcumin can be optimized.

The solid lipid nanoparticles are aqueous dispersions in which the liquid oil is substituted with a solid biocompatible and degradable lipid matrix [9]. They are characterized with higher stability (as compared with liposomes), tolerance and ability of encapsulation of many hydrophobic drugs, protected by physiological enzymes with possibility of different routes application [9]. Their small size and high surface area make them suitable for systemic administration [10].

In a recent study, free curcumin was tested as a potent monoamine oxidase inhibitor in comparison with curcumin encapsulated in solid lipid nanoparticles, prepared by micro-emulsifying technique. The results obtained showed improved bioavailability and much higher antidepressant effect of curcumin loaded solid lipid nanoparticles in treated mice, compared to free curcumin [11].

Another study reported on the potential of curcumin loaded SLN for treatment of asthma, tested in ovalbumin induced allergic model asthma in rats *in vitro*. The main mechanism to attenuate the development of asthma is blocking the NFκB protein as shown elsewhere [12]. Curcumin loaded SLNs, prepared by solvent injection method were characterized with an average size of 190 nm and negative zeta potential, suitable for systemic application. They showed much higher plasma concentration of curcumin into the lungs and liver, compared with the free agent, with potential of significant inhibition of the cytokines, especially IL-4 and IL-2 [12], which makes them promising nanocarriers for delivery of curcumin.

Arora et al. showed that curcumin loaded solid lipid nanoparticles significantly attenuated the pain and immunomodulatory cascade in rats with induced rheumatic arthritis, in comparison with the free drug [13].

The cytotoxicity study performed by MTT test of free and loaded into solid lipid nanoparticles curcumin showed significantly elevated uptake of encapsulated curcumin in IMR 32 neuroblastoma cell lines *in vitro* compared with free agent. The tested nanoparticles were prepared by high speed homogenization technique. They are characterized by spherical morphology and size below 200nm [14].

The increased bioavailability of curcumin loaded solid lipid nanoparticles has been also reported [15]. Cytotoxicity enhancement of encapsulated curcumin was presented on different human cancer cell lines: HL-60, A549, and PC3. A reduction of IC_{50} values by 54-85% compared to the free drug was observed [15].

Curcumin as a free powder and incorporated in solid lipid nanoparticles was tested as a chemopreventive agent topically administrated in mice with induced skin cancer. The results indicated significant reduction of malondialdehide by curcumin loaded nanoparticles than in mice treated with free curcumin [16].

A recent study has shown that the brain delivery of curcumin loaded SLN is improved, proved by enhanced cognition and acetylcholine esterase inhibition in rats with cerebral ischemia, associated with significant increment of the level of different enzymes such as glutathione, superoxide dismutase, catalase and mitochondrial complex enzyme [17].

Curcumin loaded solid lipid nanoparticles with enhanced bioavailability have been prepared by Kahkar et al., for testing the improvement of the behavior and also in biochemical and histochemical changes induced by $AlCl_3$, administrated in mice. The results showed considerable recovery of membrane lipids and acetylcholine esterase in comparison to the free agent [18].

However, besides the presented advantages as nanocarriers, solid lipid nanoparticles are characterized with some limitations like initial burst drug release, tendency of gelation and unpredictable particle growth which is a significant limitation for their application as nanodelivery vehicles [19].

Nanoemulsions as platforms for delivery of curcumin

Nanoemulsions are defined as thermodynamically stable homogenous mixture, containing oils or fats dispersed into the aqueous continuous phase, containing emulsifiers and surfactants, approved for human administration by FDA. Usually, the droplet size of nanoemulsions is within 20-200 nm range, with great potential for hydrophobic drug encapsulation. Many research studies have reported nanoemulsions as perspective nanocarriers used in cancer treatment, diagnostics and transdermal application [20].

The hydrophobic nature of curcumin is ideally suited for encapsulation in nanoemulsions. Thus curcumin loaded nanoemulsions showed improved oral bioavailability and suppressed NFκB activity *in vivo* compared to curcumin in the aqueous phase [21].

Significant cytotoxic effects of curcumin loaded lipid nanoemulsions on mouse melanoma and human leukemic cells has been reported [22]. The curcumin nanoemulsion was prepared by thin film hydration method, using Tween 80 as a co-surfac-

tant. The physicochemical characteristics showed particle size in the 50 - 75 nm range, suitable for systemic delivery [22].

Nanoemulsions prepared by self-nanoemulsification method exhibited significant improvement in transdermal permeability of curcumin, compared to the free drug [23]. Glyceryl monooleate and Cremophor RH_{40} were used as oil phase. Increased stability of incorporated curcumin and protection from chemical degradation has been also reported in that study [23].

An intriguing approach to overcome chemotherapy resistance of human ovarian adenocarcinoma cells is using nanoemulsions simultaneously loaded with curcumin and paclitaxel [24]. Effective delivery of both drugs into tested cells was observed associated with enhanced cytotoxicity and sensitivity to chemotherapy of the treated cells [24].

Similar results were obtained in another research study, where nanoemulsions loaded with curcumin and etoposide are described [25]. Resulting nanoemulsions were characterized with an average size below 150 nm and high entrapment efficiency, adequate for systemic application. The intracellular concentration in treated prostate cancer cells was optimized and also the pharmacokinetic profiles of the two agents when encapsulated in nanoemulsions were improved [25].

Unfortunately, nanoemulsions have some major disadvantages and limitations, such as expensive formulation, strong stability dependence on pH and temperature, high concentration of surfactants and co-surfactants, which is the challenge for searching alternative nanocarriers for drug delivery [26].

Liposomes as platforms for delivery of curcumin

One of the most promising classes of nanoscale carriers for delivery of curcumin are liposomes. They represent highly-organized spherical structures, consisting of concentrically situated phospholipid bilayers (lamellas) including water volume, between them, and also in the central cavity. Liposomes were discovered in 1961 by Bangham [27]. Their size ranges from 20 nm to several μm, whereas the thickness of the membranes from 4 to 7 nm [28]. The liposomes possess unique characteristics, owing to the amphiphilic character and low toxicity, which makes them suitable for encapsulation of, both, hydrophilic and lipophilic substances. However, the route of administration and also the encapsulation efficiency of curcumin are highly dependent of type of phospholipid in the liposome. The most often used constituents of liposomal membrane are natural phospholipids or lipids such as 1,2-dipalmitoyl-sn-glycero-3-phosphatidyl choline (DPPC), 1,2-disearoryl-sn-glycero-3-phosphatidyl choline (DSPC), egg phosphatidyl choline (EPC),dimyristoylphosphatidyl choline (DMPC), soybean phosphatidylcholine (SPC), hydrogenated soybean phosphatidylcholine (HSPC) etc. and cholesterol. Various formulations are collected in Table 1.

A number of studies have shown optimization of the pharmacokinetic of curcumin by its incorporation in liposomes [29-32]. It was proven that, treatment of rats afflicted with pancreatic cancer with curcumin loaded DPPC liposomes lead to significant life prolongation compared to animals treated with ethanol solution of curcumin. The same research showed three-fold increment of bioavailability of liposomal curcumin compared to the application of the same amount of free drug [3]. Another *in vivo* research showed more than 74% prolongation of the life of mice with implanted tumor after treatment with liposomal curcumin compared to non-treated animals.

Encapsulation of curcumin into liposomes showed dose dependent increment of fraction of apoptotic cancer cells and suppression of the activity of the NFκB [35]. A study, conducted by Kunwar et al., showed increased cell internalization of liposomal curcumin compared to curcumin loaded in albumin associates in lymphocytes and EL4 lymphoma cells, which indicated, that liposomes are capable of inner cell delivery to a greater extent than serum albumin [36].

The advantages of liposomes as nanoscale platforms for delivery of curcumin, as mentioned above, are indisputable. However, one of the main technological problems, limiting the clinical realization of liposomal curcumin is the low entrapment capacity, owing to the localization of the curcumin molecules primarily in phospholipid membranes, associated with membrane disintegration above certain concentration. This limitation is a driving force towards searching of different methods for increment of the entrapment capacity of liposomes.

Macrocyclic cavitands

In spite of the large diversity, the most researched macrocyclic cavitands as drug delivery systems are cyclodextrines and in the recent years calix[n] arenes. The main mechanism by which these macromolecules improve the pharmacokinetic characteristics of hydrophobic drugs is by specific 'host-guest' interactions.

Table 1. *Liposomal formulations of curcumin*

Formulation	Indications
DMPC:DMPG:CHOL:curcumin	Inhibitory effect on LCL proliferation, Con A-stimulated human lymphocyte and splenocyte, suitable for intravenous application [29]
DMPC:Curcumin	Suppression of growth of pancreatic carcinoma and inhibition of tumor angiogenesis *in vivo* [30]
DMPC:DMPG:Curcumin	Suppression of Colo205 and LoVo Tumor Growth in a murine xenograft model [31]
EPC:CHOL:Curcumin	Inhibition of the growth of B16BL6 melanoma cells suitable for transdermal application [32]
DPPC:CHOL:Curcumin	Cytotoxic effect on KG-1 and RPMI-8226 in vitro, suitable for systemic application [33]
Lecithin:CHOL:Curcumin	Inhibition of radiation pneumotitis. Suitable for systemic application [34]
SPC:CHOL:Curcumin	Inhibition of the growth of B16BL6 melanoma cells suitable for transdermal application [32].

Cyclodextrins

Cyclodextrins are one of the most researched macro-cycle cavitants. They represent cyclic oligosaccharides, consisting of D-(+) glucopyranose units, linked through α-(1, 4) glucosidal linkages. Depending on the number of the glucopyranose residues in the molecules, cyclodextrins are divided into three groups: α, β and γ-cyclodextrins containing respectively 6, 7 and 8 glucopyranose units.

In the last years, an interesting method of optimization of the systematic delivery of curcumin was its inclusion in cyclodextrins. Not only did the obtained inclusion complexes increase the solubility of curcumin, but also showed potent antitumor effect *in vitro* as well as optimized pharmacokinetic profile *in vivo* [37-39]. It has been shown that the formation of inclusion complex curcumin:β-cyclodextrin led to 31 fold increment of water solubility and improved the stability by more than 18% [40]. Rachmawati et al. showed that complexes of curcumin with β-cyclodextrin were characterized with two-fold higher transdermal permeability in comparison to free curcumin [41]. Antiproliferative and anti-inflammatory activity of the curcumin as a result of suppression of the THF-induced activation of NF-κB was significantly higher in curcumin, included in cyclodextrins in comparison with the free agent. The inclusion complexes have displayed significantly higher half-life and higher cell internalization [42]. Oral application of curcumin:cyclodextrin inclusion complex led to noticeable suppression of tumor growth in mice with lung cancer *in vivo*[43]. Recently, hybrid nanoplatforms were developed, in which curcumin is incorporated not only in liposomal membrane but also in the aqueous cavity as an inclusion complex curcumin:cyclodextrin [38, 44]. In particular, curcumin loaded liposomes composed of DPPC and DMPG in a 1:1 molar ratio were prepared by thin film evaporation method. The encapsulation efficiency of curcumin into conventional liposomes was significantly low only 30%. In order to improve encapsulation efficiency, curcumin was additionally added into the aqueous cavity of liposomes as a form of water soluble inclusion complex with γ-cyclodextrins by hydration of thin liposomal film. As a result the encapsulation efficiency increased almost 2 times in comparison with curcumin loaded liposomes. An average diameter of 100 nm, suitable for systemic application was determined by DLS and cryo-TEM. The cytotoxic activity of the free agent in DMSO solution and loaded into liposomes and hybrid platforms was evaluated on human cancer cell lines KHOS, MCF-7 and skin fibroblast. The results from the *in vitro* studies showed significant increase of the antitumor activity of curcumin against KHOS cell line stemming from the osteosarcoma and MCF-& tumor breast cells [44].

One of the main drawbacks of the cyclodextrins, especially γ-cyclodextrins is their relatively low aqueous solubility (18 g/L), which creates difficulties in their development as carriers of curcumin. Another problem is the expensive and long process of partition of the main types of cyclodextrins in the enzyme dissolution of the starch. Frequently, the use organic solvents such as toluene or acetone is required, the removal of which is difficult, and also the presence of these solvents is related to the emergence of unwanted immunological or toxic effects. Therefore, a research line for other macrocyclic candidates is open for contemporary pharmaceutical science.

Calix[n]arenes

An interesting group of potential candidates for delivery of curcumin are calix[n]arenes, containing phenol units linked via methylene groups in 2,6 position. These macrocycles have a 'cup'-like structure consisting of well-defined lower and upper rims and a hydrophobic cavity, suitable for incorporation of guest molecules and ions. The main limitation is related with extremely low aqueous solubility. In order to improve this unfavorable characteristic, we have recently reported of synthesis and detailed physico-chemical characterization of a series of octopus-shaped polyoxiethylated calyx[4]arenes [45]. These macrocyclic cavitands contain four PEO chains attached to the lower rim (Figure 1). Their size and size distribution were suitable for systemic application and also no evidence of cytotoxic, hemolytic and immunologic activity was found [45].

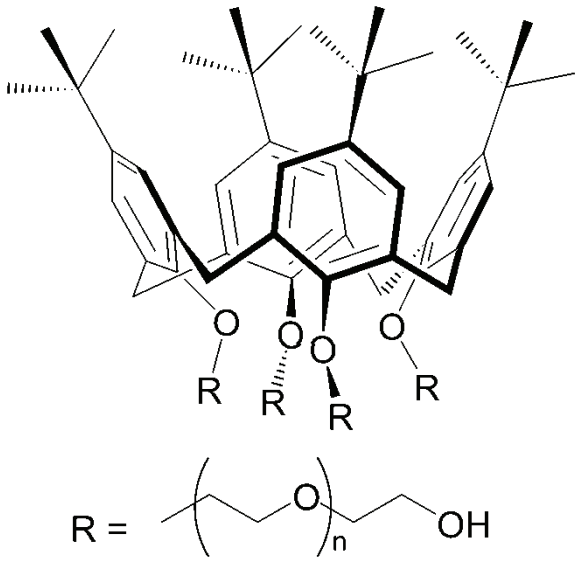

Fig. 1. Chemical structure of polyoxyethylated tert-buthylcalix[4]arene.

Above certain critical concentration, these macromolecules self-associate in water to form supramollecular aggregates. As platforms for delivery of curcumin these supramolecullar aggregates showed significant improvement in water solubility of curcumin. Unfortunately, curcumin release profile from supramolecular aggregates exhibited an initial burst effect. Therefore, in order to overcome this limitation, and also to combine the advantages of liposomes as nanocarriers, curcumin we sought out to incorporated curcumin both in the DPPC:CHOL liposomal membrane and in the aqueous cavity of liposomes as an inclusion complex curcumin:polyoxyethylated calix[4]arene [33]. Two methods were used for preparation of the inclusion complex, namely: solvent evaporation and heating method. Because of its hydrophobic nature, curcumin can be incorporated into the liposome bilayer, but, unfortunately, in limited concentration due to the destabilization effect on the liposome membrane. Therefore, the critical parameter for liposomes as perspective nanocarriers for curcumin was to evaluate the phospholipid content of DPPC:CHOL membrane for the active agent. The obtained results presented optimal molar ratio curcumin to DPPC 0.1:1, attributed with almost 90% of phospholipid recovery. The achieved encapsulation efficiency at this molar ratio was nearly 100%, but, unfortunately, the loading capacity was extremely low, only 9%. So, in order to improve the loading capacity, curcumin was encapsulated into the aqueous liposome cavity by thin film hydration with the inclusion complex curcumin:polyoxyethylated tert-butylcalix[4]arene. Obtained results showed two-fold increment of loading capacity of the hybrid platforms for curcumin. Size and size distribution of the hybrid curcumin loaded platforms determined by DLS, showed size lower than 200 nm followed with negative zeta potential suitable for systemic application. Curcumin release profile was also investigated. Results showed controlled curcumin release, without observed initial burst effect obtained in the release profile of curcumin from supramolecular aggregates. Cytotoxic activity of free and encapsulated curcumin was performed with MTT test on two human tumor cell lines: KG-1 and RPMI 8226 after 72 hours exposure. Cytotoxic effect of curcumin loaded in hybrid platforms was significantly higher, up to 6 times lower value of IC_{50} in comparison with free agent [33].

Conclusion

It is a well-known fact that the pharmacological effect of curcumin is directly associated with

its concentration in the target cells. The concentration of curcumin is dependent on the absorption, distribution and elimination. These processes can compromise the pharmacokinetics of the therapeutic agent, which leads to the necessity of application of higher dosages, and accordingly undesired immunologic and toxic effects. It is demonstrated that the encapsulation of curcumin in nanocarriers solves the problems of low solubility and instability in blood circulation and significantly improves the bioavailability of this efficacious agent. A variety of lipid based nanocarriers such as solid lipid nanoparticles, nanoemulsions, liposomes and macrocyclic cavitands such as cyclodextrins and calix[4]arenes and their liposomal hybrid systems described in this review show a profound improvement of the pharmacokinetic profile of curcumin. However, to translate curcumin nanoformulations as drug candidate allowing full utilization of its therapeutic potential, future pre-clinical and clinical investigations in depth are required.

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