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Curcumin: *In-Vitro* Anticancer Activity and Novel Drug Delivery Systems

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ABSTRACT

Curcumin has been identified as a major constituent in turmeric. It has been used in traditional medicine for liver disease (jaundice), indigestion, urinary tract diseases, rheumatoid arthritis, and insect bites. Numerous studies have demonstrated the *in-vitro* anticancer effect of curcumin. Curcumin inhibits several different cellular targets like nuclear factor- kappa B (NF-κB); this in turn induces apoptosis and blocks the function of protein kinase C. The major problem associated with curcumin is its poor aqueous solubility resulting in its poor bioavailability at the tumor site. Therefore, improvement in stability, solubility and bioactivity is needed. The current review discusses the activity of curcumin on various types of cancer along with the advances in the drug delivery systems designed to improve curcumin delivery. These newly developed formulations improve the drug bioavailability and effectiveness. Numerous formulations of curcumin have been developed like microcapsules, β-cyclodextrin inclusion complexes, nanoparticles and others such drug delivery systems.

Keywords: Aqueous solubility, curcumin, cellular targets, nanoparticles, types of cancer

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INTRODUCTION

For over 4000 years turmeric is used in Ayurveda and Chinese medicine for treatment of various ailments. It is obtained from dried roots of *Curcuma longa*, which belongs to *family: Zingiberaceae*. Curcumin, active principle component of turmeric is diferuloylmethane, a hydrophobic polyphenolic compound. Curcumin is the most abundantly occurring natural analogue at 77% [1,2], followed by demethoxycurcumin (17%) in which one methoxy group is absent, then bis-demethoxycurcumin (3%) in which the methoxy group is absent from both the aryl rings. Curcumin is a multi-target pleiotroplic agent, exhibiting a broad range of biological activities[3]. It has been used in traditional medicine for liver disease (jaundice), indigestion, urinary tract diseases, rheumatoid arthritis and insect bites. It has been observed in many scientific studies that curcumin has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity. The nature of curcumin is hydrophobic polyphenolic. As a result it suffers poor bioavailability problem resulting in submaximal plasma concentration required to exert the potential therapeutic action. The delivery of curcumin is a great challenge. As the dose cannot be increased so proportionately some effective methods of drug delivery have been designed.

Literature states studies on anticancer activity of plain curcumin administered parentrally in animals. The results indicated supression of tumor growth. Thus it has been proven that curcumin has anti-cancer effect in animals. But for these animal studies, curcumin was solubilized in organic solvent due to its poor aqueous solubility [4]. In order to develop its safe pharmaceutical dosage form it is much needed that it should be formulated using pharmaceutically acceptable excipients. This can be achieved by using novel drug delivery systems such as microcapsules, cyclodextrin inclusion complexes, lipoidal drug delivery systems and naoparticles.

Anticancer activity of curcumin

Last few decades extensive research is carried out on the life threatening disease cancer. It is being suggested that the chemopreventive is better than chemoprotective therapy. In chemopreventive therapy the body is exposed to a low toxic compound as against in chemoprotective where large amount of toxic components are introduced in body. Curcumin has been reported as chemopreventive agents for many types of cancer such as lung, skin, bladder, brain tumor etc.

Bladder tumor:

Human bladder tumor cell lines T24, UMUC2 and EJ were cultured in RPMI 1640 medium. Curcumin (98% purity) was dissolved in dimethylsulfoxide (DMSO) as a 10 mg/ml stock solution and stored at -20^{o}C. Cancerous cells were treated with 0–40 µmol/L of curcumin and incubated for 24-48 hours. The fraction of cells surviving was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. It was observed that *in vitro* the cancer cell viability was decreased in dose and time dependent manner [5].

Prostate cancer:

Prostate cancer is one of the most prevalent diseases in North America. The incidence of prostate cancer is much higher in males in Western countries (≥60.6 cases per 100,000) compared to Asian countries (<5–10 cases per 100,000) [5, 6]. The average age of 72-74 years are majorly affected by it. Studies are carried out to find better preventive majors. Curcumin was explored for its activity against androgen-dependent prostate cancer cell lines, LNCaP and the androgen-independent DU145 cell line. It was noted that curcumin was able to suppress the expression of the LNCaP cancer cell line. It was found to be effective in downregulating the androgen receptor and androgen receptor related cofactor. It acted by inhibiting the prostate specific antigen (PSA) a critical marker of prostate cancer progression, upregulated in androgen-independent prostate cancer, through the activation of nuclear factor kappa-light chain-enhancer of activated B-cell (NF- κB). Curcumin influenced multiple growth factor signaling pathways. Curcumin has been shown to both downregulate the expression of epidermal growth factor receptor (EGFR) and inhibit EGFR tyrosine kinase activity. Vascular endothelial growth factor (VEGF) was a major mediator of angiogenesis, so inhibitors of VEGF are being explored as treatments for prostate cancer. Curcumin was known to inhibit the VEGF and angiogenesis [8].

Glioblastoma:

Glioblastoma (GBM) is an aggressive, invasive and difficult to treat primary brain tumor. Standard therapy includes surgical resection, external beam radiation and chemotherapy, with no known curative therapy. A number of deregulated signaling cascades have been described in GBMs, including the nuclear factor kappa-B, the phosphoinositide-3-kinase (PI3K/Akt) pathway and the Ras/MEK/ ERK mitogen-activated protein kinase pathway. Curcumin effect occurred in both adhered and agar growing GBMs, evidencing an anchorage-independent mechanism. In addition, the antiglioblastoma activity of curcumin was prolonged and persisted for several days after drug withdrawal as assessed by clonogenic assays. Besides cell death induction at cytotoxic levels, low concentrations of curcumin decreased proliferation and migration and synergized with classical anticancer drugs, suggesting that subtoxic concentrations of curcumin also could

be taken for therapeutic advantage. Curcumin appeared to act irrespective of the different mutations. Curcumin suppressed NF-κB and PI3K/Akt, which are two pivotal survival pathways in GBMs. The arrest in the G_2/M phase as an early step of the apoptotic mechanism also could contribute to curcumin selectivity, since cancer cells are in constant cell cycle progression through S to G_2/M phase, in contrast to nonproliferative cells. Despite aggressive neurosurgery and chemotherapy, GBMs frequently exhibit chemoresistance. Identifying novel strategies to overcome drug resistance may aid in the development of improved therapeutics [4].

Angiogenesis:

One common essential factor for all types of cancer is the requirement of a suitable blood supply. Therefore, tumor vasculature has emerged as a potential target for therapeutic intervention. New blood vessel growth from preexisting vasculature stimulated by biochemical signals is termed angiogenesis. Tumor masses require a constant supply of oxygen and nutrients, and a means of efficient waste removal to ensure sustained development. Diffusion from nearby capillaries can supply adequate nutrition for tumors less than 2 mm in size, but for continued growth the tumors must develop their own blood supply. Alteration of the delicate balance of angiogenic stimulating factors and angiogenic inhibitors results in the phenotypic change from quiescence to active endothelial proliferation. To date, this angiogenic switch is not completely understood. The goal of antiangiogenic therapy is to interfere with these mechanisms and prevent tumor cells from developing a viable blood supply. Curcumin has shown to have antiangiogenic properties in vitro and in vivo [9].

Head and neck squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common form of cancer worldwide and represents approximately 5% of all cancers diagnosed annually in the United States. Studies of curcumin in various head and neck cancer cell lines have demonstrated decreased cell growth and survival, concomitant with the compound's effects on molecular pathways involved in cellular proliferation. Expression of constitutively active NF-κB and IκK (enzyme complex involved in propagation response to inflammation) has been observed in multiple oral squamous cell carcinoma cell lines, and curcumin treatment was shown to suppress growth and survival of these cell lines via inhibition of NF-κB activation [10,11]. Signal-transducer-and activator-of-transcription-3 (STAT3) is a signaling protein observed to be over expressed in multiple head and neck cancers, and curcumin was shown to suppress the interleukin (IL-6) mediated phosphorylation of STAT3 as well as inhibiting nuclear localization [12].

Cell surface binding inhibition:

It has been shown that curcumin blocks the cell surface expression of adhesion molecules in endothelial cells, and this accompanies suppression of tumor cell adhesion to endothelial cells [13]. It has been demonstrated that downregulation of these adhesion molecules is mediated through downregulation of NF-κB activation. Curcumin can modify cell receptor binding [14]. Curcumin-treated B16F10 melanoma cells formed eight-fold fewer lung metastasis in C57BL6 mice than untreated cells. It inhibited the binding of fibronectin, vitronectin, and collagen IV to the extracellular matrix (ECM) proteins. It also suppressed the expression of α5β1 and α5β3 integrin receptors, pp125 focal adhesion kinase (FAK), tyrosine phosphorylation of a 120-kD protein, and collagenase activity. Curcumin enhanced the expression of antimetastatic proteins, TIMP-2, nonmetastatic gene 23 (Nm23), and E-cadherin [13].

Osteosarcoma:

The anticancer potential of curcumin against osteosarcoma (OS) has not been fully explored and is of potentially significant impact in drug delivery. OS usually develops during the period of rapid growth in adolescence, and it occurs as a second malignant neoplasm due to a genetic predisposition and/or as a consequence of prior cancer therapy [15]. The incidence rates in the United States peak in adolescence and in elder years and accounts for about 60% of primary malignant bone tumors diagnosed in the first 2 decades of life. The prognosis for OS is poor, with a survival rate of 62% at 5 years (ages 0 – 24) for localized OS, and worse for metastatic disease situations [11]. The preferred mode of therapy is tumor removal followed by chemotherapy or radiation therapies that are nonspecific and toxic to normal cells. One of the limitations of current therapeutic options is the lack of specific therapy with fewer side effects. Therefore, there is a need to develop novel and less toxic therapeutic options to increase the cytotoxic effects. Walter *et al* have reported curcumin's cytotoxic effects on OS cell lines [16] .And they were first to report liposomal curcumin delivery for osteosarcoma.

Radiosensitizing activity:

Chendil and coworkers investigated the radiosensitizing effects of curcumin in p53-mutant prostate cancer cell line PC-3. When compared with cells that were only irradiated, cells treated with curcumin at 2 and 4 μM concentrations in combination with radiation showed significant enhancement of radiation-induced clonogenic inhibition and apoptosis. Radiation caused up-regulated TNF-α protein in these cancerous cells, leading to an increase in NF-αB activity and induction of Bcl-2 protein. Curcumin, in combination with radiation, inhibited TNF-α-mediated NF-αB activity, resulting in bcl-2 protein downregulation. These results suggested that curcumin

is a potent radiosensitizer, and it acts by overcoming the effects of radiation induced prosurvival gene expression in prostate cancer [17].

Colon cancer:

HCT-116 colonocytes were exposed to curcumin at concentrations of 10 µM causing DNA damage in the form of single-strand breaks [18]. The novel finding in this study was that curcumin caused increased expression of growth arrest and DNA damage gene (GADD153) that has been implicated in apoptosis possibly through the modulation of protein kinase C (PKC) [19-21]. Sustained activation of c-Jun N-terminal kinases (JNK) by curcumin at concentrations of 35 µM led to apoptosis of HCT116 cells accompanied by p38 activation and NF-κB inhibition[22]. Recently, it has been demonstrated that curcumin (100 µM) in HCT 116 cells leads to an increase in ceramide generation appearing to peak at the induction of apoptosis (50 µM) [23]. These findings suggested that curcumin can induce apoptosis by the production of reactive oxygen species and downstream activation of JNK and to a lesser extent by ceramide generation. Curcumin was shown to activate caspases 9, 3, and 8 in the colon cancer cell lines SW480 and SW620 [24].

The major hurdle in practical use of curcumin is its low bioavailability following oral administartion it is rapidly metabolised into its glucoronate or sulphated conjugate which are more soluble and excreated easily. The researchers are still finding whether the metabolites are active or not [25]. Phase I clinical trials of curcumin were carried out by Hsu CH and Cheng AL. They suggested that curcumin with doses up to 3600- 8000 mg daily for 4 months did not result in discernible toxicities except mild nausea and diarrhea. Curcumin *in-vitro* has measurable anti-cancer effects; while human it has to be proven. To surmount the low bioavailability, the novel drug delivery systems of curcumin are essential [26].

DELIVERY OF CURCUMIN TO CANCEROUS CELLS

MICROCAPSULES DRUG DELIVERY SYSTEMS:

Hollow microcapsules fabricated by layer-by-layer assembly (LbL) using oppositely charged polyelectrolyte have been reported by Manju *et al*. A fair amount of active component of poor aqueous solubility can be loaded into these hollow microcapsules. Melamine formaldehyde (MF) templates were alternatively coated with six double layers of poly (sodium 4-styrene sulfonic acid) (PSS) and poly (ethylene imine) (PEI). Hollow microcapsules were obtained by dissolving the MF cores using hydrochloric acid (pH <1.5). After core dissolution, the content was collected by centrifugation and the collected capsules were washed thoroughly with water. The

microcapsules were found to be cytocompatible while the extract of capsules loaded with curcumin showed severe cytotoxicity on the mouse fibroblast cell indicating released curcumin was active. The high stability of polyelectrolyte microcapsules in the aqueous medium depicted that these drug carriers were suitable for drug delivery applications [27].

NANOPARTICULATE DRUG DELIVERY SYSTEMS (NPs):

Nanotherapeutics, a hot-spot in the field of medicine, especially nanoparticle based drug delivery for cancer therapy is spreading rapidly which can overcome the limitations of conventional drug delivery systems. Nanometric drug carriers of optimum size and surface characteristics are highly stable and possess high carrying capacity. Moreover the feasibility of incorporation of both hydrophilic and hydrophobic substances and feasibility of variable routes of administration allow controlled drug release from the matrix and improved drug bioavailability.

Polymeric nanoparticles

Chitosan, a deacetylated form of chitin is a naturally occurring linear biodegradable polysaccharide and it is made up of N-acetyl-d-glucosamine and d-glucosamine. Dextran sulphate is also a biocompatible polyanionic polymer. It is a highly branched polysaccharide with 1–6 and 1–4 glycosidic linkage with approximately 2 to 3 sulphate groups per glucosyl unit. Using these two unique polymers dextran sulphate–chitosan NPs were formed. Curcumin loaded dextran sulphate–chitosan NPs were formed immediately due to the coaccervation reaction between two oppositely charged polyelectrolyte polymers, i.e. dextran sulphate (negatively charged) and chitosan (positively charged). The coaccervation reaction results from the electrostatic interaction between the protonated amino groups of chitosan and sulphate groups of dextran sulphate. The size and surface charge of the prepared NPs was tunable by varying the polymer concentration and reaction conditions. These nanoparicles had good stability and did not require any stabilisers. Curcumin loaded dextran sulphate–chitosan nanoparticles were obtained by the addition of ethanolic curcumin to chitosan solution before cross-linking with dextran sulphate. The prepared nanoparticles had a spherical morphology with negative zeta potential and good colloidal stability. In this study 70% of curcumin was released upto 120 h. These results suggested that dextran sulphate–chitosan NPs could be used as an ideal carrier to deliver hydrophobic drugs like curcumin in cancer drug delivery [28].

Delivering the drug in nanosize to the desired target site with the help of biocompatible and biodegradable polymer has evolved through the decade. Alginate (AG) and chitosan (CS) are widely used biopolymers. They are capable of undergoing polyelectrolyte complexation. AG-CS nanoparticles were used to design the controlled release formulation. Since both AG and CS are

hydrophobic in nature Pluronic F127 was introduced to facilitate the encapsulation and dispersion of curcumin. It also helped in effective passive targeting and prolonging the circulation half life of the curcumin. Curcumin release was observed to be both swelling and diffusion controlled [29].

O-Carboxy Methyl Chitosan Nanoparticles (O-CMC Nps) were prepared by the ionic cross-linking reaction of O-CMC with $CaCl_2$. Curcumin was effectively loaded into them. Curcumin-O-CMC Nps were tuned within the optimum size range suitable for drug delivery application. *In vitro* drug release profile indicated slow, controlled and sustained release of drug from the nanoparticle matrix and also demonstrated enzyme triggered degradation and release of the drug in the presence of lysozyme. Fluorescence microscopy confirmed the cellular uptake of curcumin-O-CMC Nps and apoptosis within the cancer cells. These preliminary studies showed that O-CMC Nps can be a promising candidate for carrying hydrophobic drugs like curcumin making it suitable for cancer drug delivery applications [30].

A novel polymeric amphiphile, MPEG–PA, was synthesized with methoxy poly (ethylene glycol) (MPEG) as the hydrophilic and palmitic acid (PA) as the hydrophobic segment. The conjugate was prepared in a single-step reaction. The conjugation was through an ester linkage, which is biodegradable showed minimal toxicity on HeLa cells. The MPEG–PA conjugate underwent self-assembly in an aqueous environment to form micelles with critical micelle concentration of 0.12 g. The encapsulation of a highly hydrophobic compound like curcumin in the nanocarrier made the drug readily soluble in an aqueous system, which can increase the ease of dosing, making intravenous dosing possible. Drug-loaded micelle nanoparticles showed good stability in physiological condition (pH 7.4), in simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 6.8). This micellar formulation can be used as an enzyme-triggered drug release carrier, as suggested by in vitro enzyme-catalyzed drug release using pure lipase and HeLa cell lysate. The utility of MPEG–PA to entrap the potent chemopreventive agent curcumin in the core of nanocarrier was found to be effective [31].

To improve curcumin's applicability in cancer therapy, researchers had encapsulated curcumin in poly (lactic-co-glycolide) (PLGA) (biodegradable polymer) nanoparticles, in the presence of poly (vinyl alcohol) and poly (L-lysine) which acted as stabilizers. The technique used for encapsulation was nanoprecipitation technique. These curcumin nanoformulations were characterized for particle size, zeta potential, drug encapsulation, drug compatibility and drug release. Encapsulated curcumin existed in a highly dispersed state in the PLGA core of the nanoparticles and exhibited good solid–solid compatibility. An optimized curcumin

nanoformulation has demonstrated two and six fold increased in the cellular uptake performed in cisplatin resistant A2780CP ovarian and metastatic MDA-MB-231 breast cancer cells, respectively, compared to free curcumin. This effect was correlated with enhanced apoptosis induced by the nanopaticulate curcumin formulation. Results of this study suggested that therapeutic efficacy of curcumin may be enhanced by such PLGA nanoparticle formulations, and furthermore tumor specific targeted delivery of curcumin can be made feasible by coupling of anticancer antibody to the NPs [32].

Song *et al* synthesized amphiphilic methoxypoly (ethylene glycol)-b-poly (ε-caprolactone-co-p-dioxanone) [MPEG-P (CL-co- PDO)] copolymers. Curcumin was successfully loaded into the MPEG-P (CL-co-PDO) micelles by a solid dispersion method with a high encapsulation efficiency (>95%). The micelles were used through parenteral route for curcumin delivery because they were monodisperse (Poly Dispersity Index < 0.15) with small particle sizes (around 30 nm) and were easy to reconstitute in water (just by manual shaking) after lyophilization. The stability of the reconstituted micelles at the room temperature depended on the curcumin loading contents and the PDO/CL ratios in the copolymers. MPEG-b-P (CL-co-PDO) copolymers were successfully synthesized by a simple ring-opening polymerization reaction. They showed higher water solubility and faster hydrolytic degradation compared to MPEG–PCL. The cytotoxicity assay indicated that curcumin-loaded MPEG-P (CL-co-PDO) micelles markedly inhibited the growth of PC-3 human prostate cancer cells in a dose-dependent manner. These results suggested that MPEG-P (CL-co-PDO) micelles would be a promising carrier for delivery of curcumin [33].

Recent studies show that inclusion of hydrophobic drugs into polymeric micelles is one of the most attractive methods of drug delivery. Amphiphilic block copolymers form core–shell nano-sized aggregates which can solubilize poorly soluble drugs and thus improve their bioavailability and protect from inactivation in biological media. Triblock copolymers of poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO) with the structure PEO–PPO–PEO that are known generically as poloxamers or Pluronic (PF) or Synperonic as the trade names, are highly surface-active compounds. Structural studies show that Pluronic copolymer can self-assemble into spherical micelle which is constructed with EO as a hydrophilic outer shell and PO as a hydrophobic inner core. Mixed micelles manifest synergistic properties. Mixed micelles increase stability and drug loading efficiency, superior to those of the individual components [34].

Curcumin loaded mixed micelles were prepared by thin-film hydration method. The mixture composed of 5-20 mg curcumin and 200 mg copolymer carriers (P123 and F68 with different proportions) was dissolved in dichloromethane. The solution was subsequently evaporated under

reduced pressure by rotary vacuum evaporation to obtain a thin film of drug/polymer, and the film was further dried over night at room temperature to remove any residual. After that, the film was hydrated in 5 mL of double distilled water (DD water). Ultrasonic Instrument was employed to disperse the film via ultrasound and form a micellar suspension. Non-incorporated crystalline drug was separated by filtration through a 0.8 µm filter membrane, and a yellow clear solution of curcumin-PF was obtained. The empty micelles were prepared according to the same procedure without dissolving curcumin. The mixed polymeric micelles, composed of P123 and F68 with the ratio at 2.05:1, exhibited higher encapsulation efficacy and drug loading for curcumin. The average size of the curcumin loaded mixed micelles was 68.2 nm. Compared with the curcumin propylene glycol solution, the curcumin-PF showed the sustained-release property. The in vitro cytotoxicity assay showed that curcumin-PF micelles presented higher cytotoxic effect on MCF-7 and MCF-7/ADR with curcumin in DMSO solution as control. Based on these results, it can be concluded that the mixed micelle formulation developed in this study may be considered as a promising delivery system for curcumin [35].

Magnetic nanoparticles:

Magnetic drug targeting, the targeting of a drug conjugated with a magnetic material under the action of external magnetic field constitutes an important drug delivery system. The strategy was to design a nanosized magneto fluorescent water-dispersible Fe_3O_4–curcumin conjugate and use its multiple ability to label target and treat the tumor cells. The conjugate possessed magnetic nano Fe_3O_4 core, chitosan (CS) or oleic acid (OL) as outer shell and entrapped curcumin, serving dual function of naturally autofluorescent dye as well as anti-tumor model drug, delivered to the cells with the help of macrophage [36].

The next generation magnetic nanoparticles (MNPs) with theranostic applications have attracted significant attention and will greatly improve nanomedicine in cancer therapeutics. Such novel MNP formulations must have ultra-low particle size, high inherent magnetic properties, effective imaging, drug targeting, and drug delivery properties. MNPs were prepared by chemical precipitation method and loaded with curcumin using diffusion method. The internalization of MNP-curcumin was achieved in concentration-dependent manner and demonstrated accumulation throughout the cell, which indicated that particles are not attached on the cell surface but internalized through endocytosis after 6 hours incubation with MDA-MB-231 breast cancer cells. The anticancer potential was evaluated by a tetrazolium-based dye and colony formation assays. Further, to prove MNP-curcumin results in superior therapeutic effects over curcumin, the mitochondrial membrane potential integrity and reactive oxygen species

generation were determined [37].

Liposomes

In the study carried out by Lan *et al*, curcumin in a liposomal delivery system was designed. The liposomal curcumin was prepared by dissolving curcumin in 50 mg/mL DMSO. The lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was dissolved in 20 mg/mL tert-butanol. The two solutions were mixed and filtered through a 0.22-μM filter for sterilization and lyophilized. The liposome thus formed would allow intravenous administration. The in vitro and in vivo effects of this formulation on proliferation, apoptosis, signaling, and angiogenesis was studied using human pancreatic carcinoma cells like BxPC-3, Capan-1, Capan-2, ASPC-1, HS766-T, and MiaPaCa2. NF-κB was constitutively active in all human pancreatic carcinoma cell lines and liposomal curcumin consistently suppressed NF-κB binding and decreased the expression of NF-κB-regulated gene products, including cyclooxygenase-2 and interleukin-8, both of which have been implicated in tumor growth invasiveness. The decrease in the levels of NF-κB, cyclooxygenase-2 and interleukin-8 was confirmed by electrophoretic mobility gel shift assay, immunoblots and enzyme-linked immunoassay respectively [38].

A cationic liposome-PEG-PEI complex (LPPC) was used as a carrier for the encapsulation of hydrophobic curcumin to give curcumin/LPPC. Curcumin/LPPC had an average size less than 270 nm and a zeta potential of approximately 40 mV. The LPPC encapsulation efficiency for curcumin was about 45%. The cytotoxic activity of curcumin/LPPC was higher in a variety of cancer cell lines, including curcumin sensitive and resistant cells, in comparison with non-encapsulated curcumin. Curcumin/LPPC treatment was able to arrest the cell cycle at the G2/M phase and induce apoptosis at a low dose by facilitating a rapid delivery of the drug into the cells. *In-vivo*, curcumin/LPPC treatment also resulted in a significant inhibition of tumor growth, which may be due to the higher accumulation of curcumin/LPPC than non-encapsulated curcumin in the tumor area. Thus, these results suggested that LPPC may serve as an effective drug carrier and as a useful anticancer tool. Anti-tumor activity was assessed on four different murine cancer cell lines: B16F10 melanoma cells, LL2 lung carcinoma cells, CT26 colorectal adenocarcinoma cells and JC breast adenocarcinoma cells. In addition, 4 different human cancer cell lines were also used: HepG2 hepatocellular carcinoma cells, A549 lung carcinoma cells, HT-29 colorectal adenocarcinoma cells and HeLa cervical cancer cells. Curcumin was reported to have antitumor activity against all the cell line. The cytotoxic activity of the curcumin/LPPC was fivefold higher than curcumin when tested on curcumin-sensitive cells and 20-fold more active against curcumin-resistant cells. Curcumin was also able to abolish the multidrug resistance

(MDR) of cancer cells by downregulating the intracellular levels of three major ATP-binding transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (ABCG2) and multidrug resistance associated protein (MRP-1). These results demonstrated that LPPC encapsulation technology was able to enhance the effects of antitumor drugs. Use of this technology may provide a new tool for cancer therapy, especially for drug-resistant cancer [39].

Certain biodegradable polymers can be used to modify the surface of liposomes. These modified liposomes are able to enhance the delivery of drugs, increase drug uptake by target cells and reduce drug toxicity with respect to non-target organs. Polyethylene glycol (PEG), a low-toxicity polymer, can be used to modify the surface of liposomes. These modifications serve to prolong the circulation of the liposomes by inhibiting phagocytosis by mononuclear phagocytes as well as by reducing the uptake of the liposomes by the reticuloendothelial system [40].

Solid lipid nanoparticle (SLN):

Curcumin was loaded into SLNs to improve its oral bioavailability (BA). Curcumin-loaded solid lipid nanoparticles (C-SLNs) were produced using a microemulsification technique. The particles were spherical in shape, with high drug entrapment and the in vitro release was predominantly by diffusion phenomenon was prolonged up to 7 days. No significant variation in particle size and curcumin content of C-SLNs was observed, upon storage, over a period of 12 months. In vivo pharmacokinetics performed after oral administration of C-SLNs and solubilized curcumin using a validated LC-MS/MS method in rat plasma revealed significant improvement (at $p<0.05$) in BA after administration of C-SLNs at all the doses with respect to curcumin-solution (C-S). Enhanced and reliable BA will help in establishing its therapeutic usefulness especially for neurodegenerative and cancerous disorders in humans [41].

In another method curcumin loaded SLNs of fatty acids (FA) were prepared with a coaccervation technique based on FA precipitation from their sodium salt micelles in the presence of polymeric non-ionic surfactants. Various acids like myristic, palmitic, stearic, and behenic were used as lipid matrix and different polymers with various molecular weights and hydrolysis grades were employed as stabilizers. Spherical-shaped nanoparticles with mean diameters below 500 nm were obtained from middle-high hydrolysis polymer while graded polymer SLNs produced diameters lower than 300 nm. Curcumin encapsulation efficiency was in the range 28–81%. Chitosan hydrochloride was added to FA SLN formulations to produce bioadhesive, positively charged nanoparticles. A preliminary study on HCT-116 colon cancer cells was developed to evaluate the influence of CU-loaded FA SLNs on cell viability [42].

Transferrin- mediated solid lipid nanoparticles (Tf-C-SLN) of Curcumin were designed to

increase photostability, and enhance its anticancer activity against MCF-7 breast cancer cells. Tf-C-SLN were prepared by homogenization method and characterized by size, zeta potential, entrapment efficiency, stability, transmission electron microscopy (TEM), X-ray diffraction (XRD) and in vitro release study. Microplate analysis and flow cytometry techniques were used for cytotoxicity and apoptosis study. The physical characterization showed the suitability of method of preparation. TEM and XRD study revealed the spherical nature and entrapment of curcumin in amorphous form, respectively. The cytotoxicity, reactive oxygen species (ROS) and cell uptake was found to be increased considerably with Tf-C-SLN compared to curcumin solubilized surfactant solution (CSSS) and C-SLNs suggesting the targeting effect. DNA analysis and reduced mitochondrial potential confirmed the apoptosis. The flow cytometry studies revealed that the anticancer activity of curcumin was enhanced with Tf-C-SLN compared to CSSS and C-SLN, and apoptosis was the mechanism underlying the cytotoxicity [43].

Gold nanoparticles (AuNPs)

The researchers have devised a simple method for fabrication of water soluble curcumin conjugated to AuNPs to target various cancer cell lines. Curcumin was conjugated to hyaluronic acid (HA) to get a water soluble conjugate (HA-curcumin). Gold nano particles (AuNPs) were generated by reducing chloroauric acid using HA-cur, which played the dual role of reducing and stabilising agent. The nanoparticles were subsequently anchored to folate conjugated PEG (PF-HA-curcumin-AuNPs). The blood compatibility and cytotoxicity were assayed. Their interaction with cancer cell lines (HeLa cells, glyoma cells and Caco 2 cells) was studied, followed by flow cytometry and confocal laser scanning microscopy (CLSM). Blood–material interactions studies showed that the nanoparticles were highly hemocompatible. Flow cytometry and CLSM results showed significant cellular uptake and internalization of the particles by cells. HA–Cur-AuNPs exhibited more cytotoxicity comparing to free curcumin. The strategy resulted in the formation blood compatible curcumin conjugated AuNPs with enhanced targeting and improved efficacy. The cytotoxicity studies apparently suggested that HA–Cur-AuNPs have enhanced potential to kill cancer cells compared to free curcumin. This observation was assigned to the increased entry of the particles into the cells and subsequent release of the highly soluble drug conjugate. Nearly 95% uptake of PF-HA–Cur-AuNPs could be due to significant concentration of folic acid on the surface. The high density of folic acid moieties on the surface could result in stronger affinity to the folate receptor on glyoma cell membrane and high efficacy of internationalization via folate receptor mediated endocytosis along with hyaluronic acid receptor. The microscopic studies clearly showed that functionalized particles were located both in the nuclei and in the perinuclear

region. These results were consistent with the cellular uptake of the nanoparticles [44].

Nanodisks

Nanodisks (NDs) are nanoscale, disk-shaped phospholipid bilayers whose edge is stabilized by apolipoproteins. Ghosh *et al* formulated NDs with the bioactive polyphenol curcumin at a 6:1 phospholipids-to-curcumin molar ratio. Atomic force microscopy revealed that curcumin-NDs were particles with diameters <50 nm and the thickness of phospholipids bilayers. When formulated in NDs, curcumin was water soluble. Fluorescence spectroscopy of curcumin-NDs provided evidence of self-quenching. Incubation of curcumin-NDs with empty NDs relieved the self-quenching, indicated redistribution of curcumin between curcumin-loaded and empty NDs. In HepG2 cells, curcumin-NDs mediated enhanced cell growth inhibition as compared with free curcumin. In a cell culture model of mantle cell lymphoma, curcumin-NDs were a more potent inducer of apoptosis than free curcumin [45].

Nanosuspension

Nanosuspension is used by pharma industry for incorporating poor aqueous solubility active. It represents a promising new drug formulation for intravenous administration in the treatment of certain cancers Curcumin's nanosuspension was successfully prepared by high pressure homogenization to improve curucmin's cytotoxicity, as well as improve its application via intravenous injection. Characterization of nanosuspension was done by morphology, size, zeta potential, solubility, dissolution rate, and crystal state of drug. The nanoparticles present were spherical in shape under transmission electron microscopy. Solubility and dissolution rate of curcumin in the form of nanosuspension was significantly increased because of small particle size and the crystalline state of curcumin was preserved to increase its stability against degradation. Superior cytotoxicity in Hela and MCF-7 cells was obtained in nanosuspension as compared with curcumin solution. The safety evaluation showed that, nanosuspension compared with the curcumin solution, provided less local irritation and phlebitis risks and lower rate of erythrocyte hemolysis [46].

POLYMER APPROACH:

A newer approach to deliver curcumin was to condense eight curcumin molecules into polymer known as curcumin polymer (Polycurcumin). If it was prepared by condensation it was toxic to cancer cell but if it was prepared by poly acetyl condensation then it was cytotoxic to ovarian cancer cell line (OVCAR-3) and breast cancer cell line (MCF-7). The polycurcumin was so designed to be stable in neutral pH while degrade into individual curcumin molecule at acidic pH. It was known that cancer cell is acidic in pH (around 6.4) as compared to normal cell. The

lysosomes are more acidic than cancer cell. The polycurcumin's endocytosis occurred into cancer cell with slow breakdown of terminal curcumin which was released to exhibit its apoptosis effect and arrests SKOV-3 cell cycle at G0/G1 phase as per the in vitro studies [47].

CYCLODEXTRIN COMPLEXATION:

It is well established that curcumin has poor solubility and stability. In order to explore the cyclodextrin carrier properties for delivery of curcumin, a self-assembly of β-cyclodextrin (β-CD) and curcumin via an inclusion complex mechanism was prepared. The technique used for complex formation was solvent evaporation. The cyclomaltoheptaose structure acts as a drug shuttle while a hydroxyl group of cyclodextrin imparts good solubility to the system. The developed self-assemblies, i.e., β-cyclodextrin-curcumin inclusion complexes, were confirmed by spectroscopy (FTIR, 1H NMR), thermal studies (DSC and TGA), X-ray diffraction (XRD) and microscopic studies (SEM and TEM). Cell proliferation and clonogenic assays demonstrated that β-cyclodextrin-curcumin self-assembly enhanced curcumin delivery and improved its therapeutic efficacy in prostate cancer cells compared to free curcumin. It was also found to retain the drug for a prolonged time and capable to enter in cancer cell [48].

Newer approach to deliver curcumin is to make the HPγ-CD-curcumin complex and incorporate them into the liposomes. The liposomes were prepared using thin film evaporation technique. The solubility of curcumin was increased from 11 ng/ml to 600 μg/ml. This approximately 10^4 fold increase in water solubility allowed curcumin to be accommodated in the aqueous phase of vesicles. Anticancer activity of different curcumin formulations was examined against 3 cancer cell lines. The cancer cell lines used were KHOS, RFOS and MCF-7. In KHOS and MCF-7, cytotoxic effects of liposomal curcumin were 2 to 4 times stronger than those observed in non-liposomal formulations. A liposomal curcumin-treated tumor showed loss of nuclei in dead cells. The cytotoxicity was measured using DeadEnd™ Colorimetric TUNEL assay which detects DNA fragmentation caused by apoptosis. The results indicated liposomal curcumin's anticancer potential against cancer of epithelial as well as mesenchymal origin. An interesting aspect was that liposomal curcumin initiated the caspases cascade that leads to apoptotic cell death in vitro in comparison with DMSO-curcumin induced autophagic cell death. Liposomal curcumin was significantly more effective than DMSO- curcumin in affecting cell viability for the KHOS OS cell line model. On the other hand, normal cells from the same mesenchymal tissue origin (MSCs) retained about 85% cell viability at liposomal curcumin concentrations lethal for KHOS, which was an indicator of the relative low toxicity of these liposomes on normal human cells. Although the KHOS IC50 values for both liposomal formulations based on curcumin levels were

similar, the amount of HPγCD-curcumin liposomes required to reach the effective curcumin level was 2 – 3 times lower than that needed using conventional liposomes simply because of the high curcumin encapsulation efficiency of HPγCD-curcumin liposomes. The same explanation applied to the IC50 values of liposomal curcumin against MCF-7. Hence the advantage of the CD-based formulation was the lower levels of liposomes that are needed to attain the same therapeutic effect [49].

CONCLUSION:

Curcumin, a component of turmeric, exhibits various health benefits. It is cost-effective and has been used for centuries without known side effects. The exhaustive research and numerous investigations carried over the last few decades suggest that curcumin has great potential in the prevention and cure of cancer. Curcumin modulates several biochemical pathways and numerous targets involved in carcinogenesis. *In- vitro* curcumin has well established its activity for various types of cancer like prostate, breast, brain, neck, bladder etc. Orally administered curcumin has poor bioavailability and tissue accumulation. Various curcumin delivery systems have been designed. These delivery systems have exhibited increased bioavailable fraction of curcumin and were more target specific and avoided toxicity. Well-controlled clinical trials of these systems are required to determine the potential of curcumin for prevention and therapy of disease.

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