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Therapeutic Potential of Curcumin and Its Nanoformulations for Treating Oral Cancer

Nanocurcumin and curcumin analogues in oral cancer

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Abstract

The global occurrence of oral cancer has steadily increased in recent years and is associated with high morbidity and mortality rates. Oral cancer is the most common cancer in the head and neck region of predominant epithelial origin (squamous cell carcinoma). Oral cancer treatment modalities include mainly surgery with or without radiotherapy and chemotherapy. Though proven effective, chemotherapy has significant adverse effects with evidence of resistance to anticancer drugs. Furthermore, in many incidents, tumor resistance and recurrence are commonly noted. Thus, there is an imperative need to identify suitable anticancer therapies that are highly precise with minimal side effects and to make oral cancer treatment effective and safer. Among the adjuvants, curcumin, a plant polyphenol isolated from the rhizome of the turmeric plant Curcuma longa, has been studied to have anti-infectious, antioxidant, anti-inflammatory, and anticarcinogenic properties. Curcumin has poor bio-availability, which has been overcome by its various analogs and nanoformulations, such as nanoparticles, liposome complexes, micelles, and phospholipid complexes. Studies have shown that the anticancer effects of curcumin are mediated by acting on multiple molecular targets like activator protein 1, protein kinase B (Akt), nuclear factor κ-light-chain-enhancer of activated B cells, mitogen-activated protein kinase, epidermal growth

factor receptor (EGFR) expression, and EGFR downstream signaling pathways, which play an important role in oral cancer pathogenesis and thereby making it a promising adjuvant treatment modality. This review aims to summarize the different novel formulations of curcumin and their role in oral cancer.

Key Words: Oral cancer, oral squamous cell carcinoma, analogues, curcumin, adjuvant therapy, nanocurcumin, curcumin nanoformulations, curcumin analogues

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Core Tip: Oral cancer has a high disease burden worldwide. Oral squamous cell carcinoma (OSCC) is the most predominant subtype of oral cancer. The majority of oral cancers present at an advanced stage with a poor prognosis. Timely diagnosis and early treatment are critical to achieving a superior outcome. Surgery is the recommended treatment for oral cancer; other treatment modalities are radiotherapy with or without chemotherapy. Among the adjuvants, curcumin has been studied for its anticarcinogenic potential in various cancers. Moreover, curcumin has been proven to modulate intracellular signaling pathways that control cancer cell growth, inflammation, invasion, apoptosis, and cell death, revealing its credibility in cancer therapy. This review aims to summarize the molecular pathways involved in oral carcinoma and explores different therapeutic interactions of curcumin and the role of novel curcumin formulations in oral cancer.

INTRODUCTION

Oral cancer, a disease of predominant epithelial origin, is the most common subtype of cancer arising in the head and neck region[1]. In 2020, globally, more than 300,000 new oral cancer cases were recorded[2]. Ninety percent of oral cancer cases are histologically diagnosed as oral squamous cell carcinomas (OSCCs) [3]. Oral cancer is

widely prevalent in developing countries of South Central Asia (India, Sri Lanka, Pakistan) and Melanesia, with a lesser disease burden in developed countries[4]. Oral cancer is the leading cause of death due to cancer in the Indian male population [5]. High incidence rates of oral cancer have been linked to alcohol consumption, tobacco smoking, betel nut chewing, and HPV infection[6]. Despite diagnostic and therapeutic advances, 5-year survival globally remains less than 50%[7].

The primary treatment of oral cancer is based on the cancer stage. Surgery is the mainstay of multimodal therapy, which includes radiotherapy and systemic treatment (chemotherapy and/or target agents)[8]. Chemotherapy has proven to increase treatment efficacy and improve overall survival but has significant adverse effects with the development of drug resistance[9]. The combination of an agent to make the available treatment more compelling is the need of the hour.

Among the adjuvants, curcumin, a phytochemical isolated from the turmeric plant, is being reported in the plethora of studies to have anti-infectious, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, thrombo-suppressive, anti-arthritic, chemopreventive, and anticarcinogenic properties[10]. Studies have shown the therapeutic role of curcumin in various cancers, including oral cancer[11]. Curcumin acts on numerous molecular targets like signal transducer and activator of transcription 3 (STAT3), activator protein 1 (AP-1), protein kinase B (PKB also known as Akt), notch1, nuclear factor κ-light chain enhancer of activated B cells (NF-κβ), Wnt, mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (EGFR) and the respective downstream signaling pathways, which are known to play key roles in oral cancer pathogenesis[10,12].

The hydrophobic nature of curcumin with poor bioavailability and sensitivity of soluble curcumin in physiological pH has limited its use in clinical practice[13]. The nanotechnology-based techniques have made possible various novel formulations of curcumin, such as liposomes, nanoparticles, micelles, phospholipid complexes, and analogs, to improve its tissue level absorption and increase its pharmacological effectiveness[14]. Studies have shown favorable results on the effect of nano-formulated

curcumin on epithelial-type cancers. Thus, it favors a therapeutic role in oral cancer treatment as an adjuvant[15-17]. The present review aims to summarize the key properties of curcumin and its novel formulations, along with their role in oral cancer.

ORAL SQUAMOUS CELL CARCINOMA

OSCCs are majorly present as non-healing ulcers or growth. Early in the disease, lesions can appear as flat, discolored areas (erythroplakia/ leukoplakia)[18]. An invasion of surrounding tissues can present as neck masses, trismus, referred ear pain, and specific sensory changes[19]. In cancer of the lip, there is often an exophytic, crusted lesion invading the underlying muscle with tissue damage in the adjacent lip[20]. Oral cancers are often diagnosed late due to an asymptomatic phase with fast progression and early metastasis[21]. Furthermore, the staging of oral cancer plays a significant role in survival rate, with early-stage (I and II) and advanced-stage (III and IV) lesions having a 5-year survival rate of 80% and 50% or less, respectively [21].

There are multiple pathways involved in oral carcinogenesis leading to genetic mutation (H-ras, K-ras), gene deletions (loss of chromosome 9p21, 3p), promoter methylation (p16, RASSF1), amplification of oncogenes and oncoproteins (EGFR, myc, bcl-2, ras, raf, stat-3, cyclin D1) and inactivation of tumor suppressor gene (p53)[22].

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PROPERTIES OF CURCUMIN

Curcumin is a yellow spice derived from the roots (rhizomes) of Curcuma longa, commonly known as turmeric[23]. Turmeric contains curcuminoids, comprising curcumin, demethoxy curcumin (DMC), and bis-demethoxycurcumin (BDMC)[24]. In 1910, the principal ingredient of curcumin was identified by Milobedzka *et al* as diferuloylmethane[25]. Curcumin is known as 1, 7-bis (4-hydroxy-3-methoxy phenyl)-1,

6-heptadiene- 3, 5-dione (1E-6E) by IUPAC nomenclature with 368.4 g/mole molecular weight and a melting temperature of 183°C[26].

Curcumin contains two aromatic ring systems with o-methoxy phenolic groups linked with α-and β-unsaturated β-diketone moiety (Figure 1) [27]. The absorption bands of curcumin exist in the visible region of the spectrum (410-430nm) and the ultraviolet region (250–270nm range)[14]. At 488nm, curcumin gets excited with a lower fluorescent yield emission in the 500-530nm range, which can be detected by flow cytometry and confocal microscopy[28]. Curcumin is insoluble in water and readily soluble in polar solvents with keto-enol tautomerism[29]. Keto-form predominates in acid or neutral solutions, with enol-form being predominant in alkaline solutions[30].

The bioavailability of curcumin has shown to be around 1% in various animal studies, suggesting a requirement of high doses of curcumin (3600 to 12,000 milligrams) to observe any beneficial effects[31]. It is a known fact that curcumin's solubility in water (0.0004 mg/mL at pH 7.3) is poor, causing apparent problems with oral administration[32]. A study has shown that Curcumin has no toxic effect in patients with colorectal cancer when its oral administration is at least 3600 milligrams[33]. Curcumin undergoes rapid metabolism in the liver and gets excreted in the feces[34]. Curcumin is transformed into dihydrocurcumin (DHC) and tetrahydrocurcumin (THC) and consequently converted into glucuronide conjugates[35,36]. In intestinal mucosa, kidney, and liver, the conjugative enzyme activity for glucuronidation and sulfation of curcumin has been discovered[36,37]. A study stated that a considerable portion of orally administered curcumin was conjugated to glucuronide in the intestine; later, the conjugated compound entered into the portal vein and underwent additional conjugation to form glucuronide/sulfate metabolites of curcumin in the liver[37].

Curcumin, having a vast range of effects on various human diseases, plays an anti-tumorigenic role in different cancers by affecting multiple pathways of cancer progression[10-12]. In addition, it has been shown to have different effects on normal cells than cancer cells, including high cellular uptake in cancer cells than in normal cells[38]. The anticancer effects of curcumin are predominantly mediated through its regulation

of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other oncogenic molecules, as summarised in Figure 2[10,11,32]. Curcumin metabolites (THC, hexahydrocurcumin, and octahydrocurcumin) also have anticancer properties[39,40]. However, the major drawback of the reduced bioavailability of curcumin is primarily due to its poor absorption, rapid degradation, fast metabolism, and systemic elimination. All these factors combinedly restrict the use of curcumin as a novel chemotherapeutic agent in cancer therapy. Notably, as an anti-cancer drug, curcumin should be administered in a high concentration where patients show intolerance to bulk doses of curcumin[32]. Using an advanced delivery system to increase the bioavailability of curcumin with satisfactory parenteral administration is the way forward in making it a promising anticancer agent in the clinical field.

EFFECTS OF CURCUMIN ON ORAL CANCER

Curcumin is a potent agent inhibiting cancer cell growth and DNA synthesis in oral cancer cells[40]. Treatment with curcumin promotes the cell cycle's G(2)/M phase arrest, accompanied by a decrease in cyclin B/ cyclin-dependent kinase 1 and CDC25C protein levels. It induces apoptosis of oral cancer cells with a reduction of the Bcl-2 Level, reduction in mitochondrial membrane potential, promotion of the active forms of caspase-3, the release of apoptosis-inducing factor, and endonuclease G from the mitochondria[41]. Curcumin and curcuminoids like DMC and BDMC have shown autophagic and apoptotic activity[42,43].

Studies have shown that curcumin significantly inhibits carcinogen activating enzyme CYP1A1 (Cytochrome P450 family 1 subfamily A member 1), which mediates benzo(a)pyrene diol bioactivation in both OSCC cells and oral mucosa[44]. Arecoline is another significant risk factor of oral cancer, and treatment with curcumin markedly inhibits arecoline-induced Snail expression[45]. A study showed that administration of curcumin at 100 mg/kg for 12 wk in the rat model with 4-nitroquinolone-1- oxide (4-NQO) induced oral cancer markedly decreased the expression of proliferating cell

nuclear antigen, anti-apoptosis markers (Bcl -2), suppressors of cytokine signaling 3 and 1, and STAT3, minimized the cellular atypia, reduced gene expression of Vimentin, E-cadherin, N-cadherin, or TWIST1 representative of epithelial-mesenchymal transition (EMT) events[46]. Combining local and systemic C3 complex (a purified mixture of curcumin, BDMC, and DMC) effectively targets cancer cell proliferation. It inhibits 4NQO-induced tumorigenesis *via* modulation of the fibroblast growth factor-2/fibroblast growth factor receptor-2[47]. Moreover, curcumin inhibits the activation and expression of host transcription factors AP-1 and NF-κB, which bind to a cis-regulatory region of the HPV genome. This effect is concentration and time-dependent, leading to the suppression of HPV16/E6 transcription and subsequent prevention of oral carcinogenesis[48]. Curcumin triggers the activation of p38, which then interacts with binding elements in insulin-like growth factor binding protein-5, leading to the activation of the transcription factor CCAAT/enhancer binding protein α (C/EBPα). This results in the suppression of oral carcinogenesis[49].

Additionally, curcumin reduces oral cancer cell viability and invasion by down-regulating Notch-1 and NF-κB[12]. It also induces G2/M phase cell cycle arrest dose-dependently by inhibiting the phosphorylation of EGFR and its downstream signaling molecules Akt, extracellular signal-regulated kinase (ERK1/2), and STAT3 [12]. Treatment of oral cancer cells with curcumin, BDMC, and DMC leads to the production of reactive oxygen species (ROS), activation of caspase-8, -9, and -3, decrease in the levels of matrix metalloproteinases (MMP), the release of apoptosis-inducing factor (AIF) causing cell apoptosis, and alteration in the expressions of EGFR, PI3K, p-AKT, NF-κB, AMP responsive protein kinase, and MAPK[43]. In the *in vivo* OSCC model, curcumin has also been observed to suppress the expression of cyclo-oxygenase-2[50].

Oncogenic microRNA, miR-31, is upregulated in OSCC, and curcumin downregulates miR-31 expression in OSCC, attenuating AKT activation and down-regulating C/EBPβ[51]. Moreover, curcumin inhibits oral cancer cell proliferation by

upregulating miR-9 expression in a dose-dependent manner and suppressing Wnt/β-catenin signaling[52]. Besides, curcumin can also enhance the antitumor immune response by inhibiting the expression of programmed cell death ligand 1 and pSTAT3 both *in vivo* and in vitro, leading to an increase in CD8+ T cells and a decrease in T regulatory cells and myeloid-derived suppressor cells[53]. Cancer-associated fibroblasts (CAFs) are 'activated' fibroblasts in the tumor microenvironment that play a critical role in cancer development[54]. Curcumin can reverse the phenotype of CAFs to that of peri-tumor fibroblasts-like cells by downregulating the expression of α-smooth muscle actin (a unique marker for CAFs) and inhibiting the secretion of pro-carcinogenic cytokines, such as transforming growth factor-β1, MMP2 and stromal cell-derived factor-1[55]. This results in decreased cancer invasion, as there is a reduced release of EMT-mediators in treated CAFs and reversal of EMT in treated tumor cells[56].

Hepatocyte growth factor (HGF) signaling plays an important role in EMT induction and contributes to cancer cell invasion and metastasis[57]. In addition, curcumin inhibits HGF-induced EMT and cell motility in oral cancer cells, acting on HGF receptor c-Met and blocking the downstream activation of the pro-survival ERK pathway[58]. It also decreases cell proliferation in cell lines with mesenchymal characteristics and causes cell death, with a dose-dependent decrease in cell–cell adhesion[59]. Curcumin treatment has been found to suppress MMP-2, and MMP-9 MMP10 expression, which is linked to cancer cell migration and invasion in oral cancer cells [60,61].

Studies have shown that curcumin can enhance the efficacy of standard platinum-based chemotherapy for treating oral cancer, resulting in significant tumor growth suppression in cell lines and mouse xenografts[62,63]. These results propose the potential of clinically using subtherapeutic doses of cisplatin in combination with curcumin to effectively suppress tumor growth and minimize cisplatin's toxic side effects.

Furthermore, curcumin has a radio-sensitizer effect in OSSC and exhibits synergistic anti-proliferative activity when combined with cetuximab (anti-EGFR monoclonal antibody) in cisplatin-resistant oral cancer cells[64,65]. A study with combinations of curcumin and metformin showed to reduce the tumor volume and improve overall survival of the animals with downregulation of cancer stem cell markers (CD44 and CD133)[66]. Another study showed that olaparib (a poly-ADP ribose polymerase inhibitor), when combined with curcumin *in vitro* and *in vivo* (mice model), causes DNA damage, inhibits cell proliferation, and topoisomerase activity, reduces the expression of base excision repair components, induces apoptosis and decreases the tumor volume [67].

CLINICAL TRIALS USING CURCUMIN FOR ORAL LESIONS

Kuriakose MA *et al* conducted a study on oral leukoplakia, a potentially malignant oral cavity lesion with no effective treatment available, where subjects with oral leukoplakia underwent a randomized, double-blinded, placebo-controlled phase IIB clinical trial with curcumin. Clinical and histological response assessment showed a significantly better outcome with curcumin treatment. Notably, the therapy was well-tolerated, and a significant and long-lasting clinical response was observed for six months after the treatment with curcumin (3.6 g for six months)[68]. Furthermore, recent studies have shown that topical curcumin effectively treats oral mucositis[69]. Currently, a phase II randomized trial (double-blind, placebo-controlled) is ongoing to assess the therapeutic effects of curcumin in patients with stage III-IV head and neck cancer and cancer anorexia-cachexia[70]

NOVEL FORMULATIONS OF CURCUMIN IN ORAL CANCER TREATMENT

CURCUMIN ANALOGUES

Several investigators have been attempting to improve curcumin's therapeutic effectiveness and pharmacokinetic profile by developing new analogs [71,72]. The synthetic curcumin analogs that have been studied for their anticancer roles include the

EF series (EF24, EF31, and UBS109), the FLLL series (FLLL11, FLLL12, FLLL31, FLLL32, and FLLL62), the GO-Y series, 4-arylidene curcumin analogs: AC17, B19 [(1E,4E)-1,5-bis(2,3-dimethoxy phenyl) penta-1,4-dien-3-one], CDF (difluorinated curcumin), 4-[3,5-bis(2-chlorobenzylidene-4-oxo-piperidine-1-yl)-4-oxo-2-butenoic acid] CLEFMA, the diarylidenylpiperidones series, DM-1 (sodium 4-[5-(4-hydroxy-3-methoxyphenyl)-3-oxo-penta-1,4-dienyl]-2- methoxy phenolate) and dimethoxycurcumin[73]. In addition, some of these new analogs have been reported to have more potent anticancer properties than curcumin and beneficial antioxidant, antimalarial, and anti-inflammatory roles[74-78].

A few analogs, such as EF24, CDF, and FLLL12, exhibit enhanced physicochemical properties like improved solubility and bioavailability, which overcome the limitations of curcumin[79,80]. In addition, some analogs have been shown to increase efficacy and overcome chemotherapy agents' resistance when combined[81,82]. These analogs have shown promising results in breast, prostate, colon, and head-neck squamous cell cancer[76,83-86]. In a study on oral cancer cells, Chuprajob T *et al* 2014 found that curcumin analogs with the 1,4,6-tried-3-one function are more potent than the curcuminoids. Also, structural variations in the analogs enhanced their potency for example – the meta-oxygen function on the aromatic ringside is more potent than those in the ortho- and para-positions, and the free phenolic hydroxy group is more potent than the corresponding methyl analogs. Furthermore, some analogs showed less toxic effects than curcumin in normal cells[87].In 2017, Lin C *et al* found that EF24 exhibited antitumor activity on CAL-27 oral cancer cells by deactivating the MAPK/ERK signaling pathway[88].

Several curcumin analogs have been developed in recent years, and most of the analogs have shown similar mechanisms of action as curcumin. Still, some have unique mechanisms that are not associated with curcumin. For instance, the B19 analog inhibits the thioredoxin reductase 1 enzyme leading to ROS-mediated endoplasmic

reticulum stress, whereas AC17 blocks proteasome by inhibiting the deubiquitinase activity of 19S regulatory particles, neither of which are seen with curcumin[89,90]. Further studies are required to evaluate the specific benefits of these inhibition pathways in cancer treatment.

Despite the promising potential of these analogs, key parameters for the clinical development of many promising analogs remain unknown, and further focus should be given to their pharmacokinetic studies. To reduce drug-associated toxicities and improve bioavailability, targeted drug delivery through formulation has been gaining attention. Some studies show that the curcumin analogs have been conjugated with homing moieties to direct their delivery and accumulation at specific sites. Certain homing moieties have been tested for delivering curcumin analogs o particular sites, like hyaluronic acid (HA) targeted nanomicelles, HA dendrimers, folic acid conjugated CDF, EF24 conjugated to coagulation factor VIIa targeted to tissue factor[91-94]. These potential agents have yet to undergo clinical trials and cost-effective production strategies. Further *in vivo* studies can pave the way for clinical trials and future applications.

NANOFORMULATIONS OF CURCUMIN

Nanotechnology has led the way in developing nanoscale drug delivery systems. Hydrophobic molecules such as curcumin can have improved bioavailability due to the surface effect, small size effect, quantum size effect, and quantum tunnel effect of nanoparticles[95,96]. Several novel strategies have been developed to design curcumin nanoparticles as targeted drug-delivery systems, and these have been studied in various disease states, including cancer[32]. Figure 3 depicts a diagram of these nanoformulations.

Following is a brief introduction to the different nanotechnology-based drug delivery modes with curcumin: –

Liposomes - These are spherical closed phospholipid vesicles that incorporate drugs in the inner aqueous layer and have been widely used to enhance the bioavailability and efficacy of curcumin. In recent years, several liposomal curcumins with polymeric conjugates have been modified to achieve better clinical outcomes[97]. Nanoliposomes have shown properties such as sustained-release drugs, enhanced tumor targeting, minimized toxicity to healthy cells, and a reduced oral dose of drugs[98].

Polymer micelles - An excellent drug-delivery system for curcumin, as they can overcome issues with poor solubility, low stability, and poor bioavailability. Encapsulating curcumin within cationic micelles like cetyltrimethylammonium bromide or dodecyl trimethyl ammonium bromide can enhance drug-loading capacity, increase water solubility, reduce toxicity, and lower degradation[99]. Nanomicelle curcumin prevents and treats oral mucositis caused by head and neck radiotherapy and chemotherapy[100,101].

Polymer nanoparticles - *Polymer nanoparticles* are another effective drug delivery system due to their high biocompatibility and ease of circulation in the bloodstream for a longer time. Synthetic polymer conjugates like chitosan, d,l-lactide-co-glycolide (PLGA), polyethylene glycol (PEG), poly (n-butyl) cyanoacrylate, silk fibroin, N-iso-propyl acrylamide, and hydrophobically modified starch are commonly used. Curcumin-loaded nanoparticles using PEG-5000 as a carrier stabilizer for PLGAs have shown results in cancer mice models with higher cellular uptake and induction of apoptosis both *in vitro* and in vivo[102].

Solid lipid nanoparticles (SLNs) - SLNs consist of natural lipids, like lecithins or triglycerides, that remain solid at 37°C. This molecule protects labile compounds from chemical degradation and improves bioavailability. Curcumin-loaded solid lipid

nanoparticles have shown enhanced cellular uptake and are promising anticancer agents in breast cancer cells in vitro[103].

Inclusion complex - They are formed of cyclodextrins, cyclic oligosaccharides composed of six to eight glycosyl monomeric units (α-1,4 Linked). They are widely used to improve stability, enhance water solubility, increase bioavailability, and reduce bitterness. β-Cyclodextrins are commonly used to form an inclusion complex with curcumin by solvent evaporation techniques or pH shift protocols[104].

Solid dispersions - Solid dispersions have improved curcumin's physicochemical and pharmacokinetic activities. Wet-melting and subsequent freeze-drying are common strategies for preparing crystal and amorphous forms, where curcumin is dispersed in an inert carrier at a solid-state[105].

Magnetic nanoparticles - Drug-loaded magnetic nanoparticles can be targeted in cancer-infected tissues under external magnetic fields, as they offer a targeted drug delivery option. Entrapping curcumin in Fe3O4- curcumin conjugate with oleic acid or chitosan in the outer shell results in the formation of nano-sized, fluorescent-magnetic, water-dispersible nanoparticles with increased cellular uptake and enhanced bioavailability[106].

Microspheres and microcapsules - The approach is to encapsulate drugs or curcumin within polymeric particles to improve efficacy and organ-targeted bioavailability such as camptothecin, rutin, zedoary oil, andrographolide, and eudragit S-100[107].

Emulsions - Microemulsions refer to small-droplet dispersions, with droplet sizes ranging from 1-100μM, of oil and water mixtures that are stabilized by surfactant molecules forming interfacial films. These are lipid-based drug delivery systems possessing numerous advantages, including thermodynamic stability, improved drug

dissolution, and increased solubility[108]. High-speed and high-pressure homogenization procedures, using triacylglycerol as oil and Tween-20 as emulsifiers, produce tiny microemulsion droplets. Incorporating curcuminoid into nanoemulsion has been shown to increase oral bioavailability, making it a promising treatment option[109].

Nanogels - Nanogels are three-dimensional polymer networks with high drug-loading capacity, high dispersion stability, targeted drug-delivery efficiency, fast drug-releasing properties, increased drug delivery across cellular barriers, and easy to modify chemically. Curcumin has been used as a nanogel for targeted therapy[110].

Nanoparticle curcumin - Nanoparticle curcumin is a pure form of curcumin that is processed into nanoparticles of ~200 nm in size without carrier conjugates. In vitro and *in vivo* studies have shown that these nanoparticles of curcumin exhibit increased cellular uptake and enhanced anticancer effects due to their size, surface charge, and surface area[111-113].

Niosomes: - Niosome nanocapsules are drug carriers composed of non-ionic surfactants that form a bi-layered structure with hydrophobic and hydrophilic parts in an aqueous medium. Niosomes offer numerous advantages, including improved pharmacokinetics, drug stability, therapeutic effects, and reduced side effects of administered drugs[114].

Using nano-formulation-based combination therapy has gained demand as a potent drug delivery system, overcoming conventional therapeutic agents' limitations. This delivery system has been shown to improve intracellular drug concentrations and enhance the synergistic activity for cancer therapy[115-117]. Specific curcumin novel formulations have been studied for their efficacy in treating oral cancer, with encouraging findings presented in Table 1.

In 2012, Lin HY *et al* conducted a study to assess the effect of curcumin microemulsion on oral cancer cell lines. They found that exposure to curcumin-containing

microemulsions for a brief period demonstrated cytotoxic effects in oral cancer cells. However, adding ultrasound enhanced these effects in OSCC-25 cells[120]. The authors reported statistically significant cytotoxicity with the ultrasound application. It is likely due to enhanced curcumin delivery to the cell by the fusion of microemulsion droplets with cell membranes or by reducing transport limitations by ultrasound-induced mixing and/or heating. These orally ingestible microemulsions can be therapeutic in concentration-adjusted dosing and have tissue-targeting properties with ultrasound. Studies have shown that curcumin nanoparticles (Cur-NPs) possess significantly greater bioavailability and water solubility than free curcumin[121,122]. In the 2013 study by Chang PY *et al* on CAL27-cisplatin-resistant human oral cancer cells (CAR cells), the water-soluble PLGA Cur- NPs enhanced the drug effect. Cur-NPs were found to increase ROS production, upregulated the protein expression levels of cleaved caspase-3/caspase-9, cytochrome c, apoptotic protease activating factor-1, AIF, and Bax, and downregulated the protein levels of Bcl-2. Cur-NPs triggered the intrinsic apoptotic pathway by regulating the function of multiple drug resistance proteins 1 (MDR1) and the production of ROS in CAR cells[102]. Previous studies have also reported that the MDR1, or permeability glycoprotein on the cell surface membrane, is a significant target protein of Cur-NPs[123,124]. In this study, CUR NPs treatment decreased mRNA and protein levels of MDR1 in CAR cells, indicating the induction of CAR cell apoptosis and demonstrating promise as a novel treatment for Cisplatin-resistant oral cancer.

Curcumin is phototoxic in the presence of oxygen[125,126]. Singh SP *et al* conducted a study in 2014 that demonstrated the use of organically modified silica nanoparticles (SiNp) as a vehicle for the delivery of curcumin in human oral cancer cells. The results showed improved uptake of curcumin and phototoxicity in cancer cells. Incubation time-dependent cytotoxicity, inhibition of NF-κB activity, suppression of NF-κB-regulated proteins involved in invasion (MMP-9), angiogenesis (vascular endothelial growth factor), and inflammation (tumor necrosis factor α) were observed with curcumin-SiNp. These results suggest that curcumin–SiNp formulation has significantly improved anti-cancer effects over free curcumin in the dark and on exposure to

light[127]. These findings are likely the result of increased oxidative stress in the cancer cells on visible light exposure in the presence of oxygen. The curcumin–SiNp formulation also enhances the stability of curcumin at physiological pH and increases its aqueous solubility

In 2015, Mazzarino L *et al* conducted a study on the effect of mucoadhesive polycaprolactone (PCL) nanoparticles coated with chitosan and loaded with curcumin as a treatment for oral cavity cancer[128]. The researchers used the nanoprecipitation method to prepare the chitosan-coated PCL nanoparticle with curcumin loading[129]. The chitosan-coated nanoparticles showed mucoadhesive properties by interacting with the glycoprotein mucin through electrostatic forces. In vitro studies showed that these novel curcumin nanoparticles significantly decreased the viability of SCC-9 human oral cancer cells by inducing apoptosis[128]. The study also suggested that drug retention in the mucosa after treatment with chitosan-coated curcumin-loaded nanoparticles could be helpful for local therapy in numerous diseases.

Gold nanorods (GNRs) are well-known for their photothermal activity and inherent tumor-targeting properties [130]. In 2018, Zhu F *et al* developed a novel system for combined plasmonic photothermal therapy and chemotherapy using the tumor microenvironment and near-infrared responsive gold nanorod-drug conjugates (Au NR@Curcumin). The researcher tested the antitumor effects of Au NR@Curcumin on human lung cancer cells, liver cancer cells, and human oral carcinoma cells and found that it showed more potent cytotoxicity than the free drug. Oral cancer cells showed cell cycle S phase arrest. The study suggested that Au NR@Curcumin could be an efficient photothermal agent to induce instant photothermal killing of the cancer cells, even at a low irradiation power density[131]. In 2020, Ghosh S *et al* developed a multimodality nanoconjugate by functionalizing the GNR surface with a cytotoxic nucleoside [5-fluoro-2′-deoxyuridine (FdU)] containing DNA hairpin followed by hydrophobic complexation of curcumin. The study showed that curcumin could be noncovalently complexed into small DNA hairpins for enhanced cellular delivery. It increased

cytotoxicity in oral cancer cells SCC 131 in combination with FdU nucleotides, demonstrating its potential for advanced cancer therapy[132].

Several studies have investigated the potential of curcumin nanoparticles in combating oral cancer. Saurabh Srivastava *et al* conducted a study in 2018 that found that Nano-CU, a curcumin nanoparticle, exhibited chemoprotective properties against 5-fluorouracil (5-FU) induced toxicity in oral cancer cells. Nano-CU was found to have an antioxidant effect and altered apoptotic proteins Bcl2 and Bax expression in the treated cells[111]. Another study by Lai KC *et al* in 2019 explained the anticancer properties of gefitinib (Gef) and curcumin-loaded nanoparticles (NPs) in human oral cancer SAS cells *in vitro* and SAS cells xenografted tumors in vivo. The results indicated that γ-polyglutamic acid-coated (PGA)- Gef/Cur NPs could be internalized into SAS cells and significantly decrease the total cell viability of SAS cells. Both free Gef/Cur and γ-PGA-Gef/Cur NPs induced apoptotic cell death *via* caspase-3, 9, and mitochondria-dependent pathways. In vivo studies showed that γ-PGA-Gef/Cur NPs significantly suppressed tumor size compared to the free Gef/Cur-treated group[133]. Essawy MM *et al* in 2022 developed nanoparticle curcumin using a more straightforward and cost-effective solvent-antisolvent precipitation technique and studied its effect on oral cancer cells. The study found promising cytotoxic results *via* apoptosis in contrast to the necrotic effect observed using doxorubicin in the cell lines. The authors also reported the observed luminescence of the nanoparticle curcumin, qualifying it as a double theranostic agent[134]. In another study, combining nanoparticle curcumin (~ 200 nm size) and cetuximab on oral cancer cells showed higher cytotoxicity than cetuximab alone[135]. The studies mentioned above highlight the potential chemo-adjuvant role of curcumin nanoparticles in combating oral cancer.

In 2019, Ferreira SBS *et al* aimed to develop nanostructured gel formulations containing curcumin for oral cancer therapy. The authors have shown that the use of this novel curcumin led to its rapid incorporation and localization in the hydrophobic portion of nanometer-sized polymeric micelles, resulting in increased retention after application in the oral cavity. Cytotoxicity testing showed that the formulation

selectively targeted cancer cells over healthy cells. Therefore, these systems may improve the physicochemical characteristics of curcumin by increasing its release and permeation and enhancing cancer cell targeting properties [136]. Recently in 2022, Fazli B *et al* found that curcumin-loaded niosome significantly inhibited the growth and necrosis of oral cancer cells compared to free curcumin. Histopathological specimens from the rat's mouth with induced cancer show that ed niosome curcumin treatment effectively and efficiently inhibited oral cancer. The authors also highlighted that the injectable curcumin-loaded niosome (for systemic use) was more effective than the mouthwash form of application (for topical use)[137]. With all the promising findings, future studies should be designed to explore the outcomes of novel curcumin formulations in pre-clinical and clinical trials.

CHALLENGES AND FUTURE DIRECTIONS

Oral cancer is a highly malignant disease with a poor 5-year survival rate and limited treatment options, highlighting adjuvant therapy's importance. Curcumin, known for its pleiotropic effects and potential therapeutic benefits, has shown promise as a treatment choice for patients with cancer. It has shown improvement in the efficacy of current cancer therapeutics, including overcoming the resistance of cancer cells to chemo-radio therapy. However, several clinical and practical challenges need to be addressed before curcumin can be incorporated into clinical practice as a drug for cancer. The purity of the curcumin compound significantly affects its activity and is of primary importance when used in studies or trials[138]. In addition, body tissue distribution and uptake of curcumin which account for its biological activity, need better understanding[30]. Clinical trials with curcumin have faced various challenges and difficulties, such as high metabolic instability, poor aqueous solubility, inadequate focalization, complex pharmacokinetic profile, and poor patient adherence [139].

Nanotechnology-based formulations and analogs have shown potential in overcoming the poor bioavailability issue of curcumin by improving its stability, increasing cellular uptake, and offering controlled release. However, these formulations

often lack tissue specificity. Although the various novel nano-formulations of curcumin show remarkable anti-neoplastic, theranostic, and chemo-adjuvant properties, there are technical challenges in drug development with the need to regulate the size of curcumin nanoparticles for drug delivery applications. In addition, these processes are mostly expensive and have yet to be commercialized. The effect of newer delivery systems, such as a nanoparticle, and liposomes, on the therapeutic efficacy of curcumin needs to be further investigated, as while they have been shown to enhance curcumin bioavailability, the possibility of off-target toxicity has not been thoroughly studied[140]. Curcumin has shown cytotoxic and cytoprotective effects at different doses and concentrations in various cancer studies[111,134,141,142]. These findings need consideration in pre-clinical and clinical trials with the newer formulations. Furthermore, the wide range of research variability in human cancer studies using these novel curcumin formulations, including differences in study design, drug design, sample size, and route of administration, also makes it difficult to compare and conclude which formulation has the best overall pharmacokinetic properties[139].

CONCLUSION

In conclusion, the encouraging findings from the various studies using novel curcumin formulations indicate the need for extensive pre-clinical and clinical research to shed light on their pharmacokinetics, biocompatibility, toxicity, and dose regimens in normal and disease conditions to incorporate them in treatment strategies. Systematic efforts need to be focused on identifying a potential curcumin formulation for clinical trials. Collaboration between clinicians, translational scientists, medicinal chemists, and pharmacologists is necessary to advance these agents toward clinical use as oral cancer therapeutics.

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