Digest Journal of Nanomaterials and Biostructures Vol. 3, No.4, December 2008, p. 163 - 169

DOWNREGULATION OF TELOMERASE ACTIVITY MAY ENHANCED BY NANOPARTICLE MEDIATED CURCUMIN DELIVERY

Vijendra Kumar Mishra*, Ghulam Mohammad[a], Shobha Kant Mishra[b]
School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi-221005, (U.P); India.
[a]*Dept. of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi-221005, (U.P); India.*
[b]*Prof. & Head of Dept. of Physics, M.K.College, L.N.M.University, Darbhanga.*

Telomerase activity is the key modulator of most of the cancer cells. Telomerase, a reverse transcriptase, has been found to be activated in more than 80% of human cancers and, therefore, can be considered as a potential marker for tumorigenesis. The ability of curcumin indicates the possibility of developing as a potential universal cancer chemoprevention and chemotherapeutic agent. Curcumin may regulate multi-targeted human therapy. The present investigation is made to review the nanoparticle-curcumin complex in the detection and regulation of telomerase activity. Nanoparticles encapsulated curcumin like nanocurcumin are one of the major means that could be used to improve bioavailability of curcumin. Nanoparticle mediated delivery of curcumin like nanoparticle encapsulated curcumin, barbicode assay**,** curcumin embedding phospholipids vesicles or lipid-nanospheres, liposome mediated curcumin drug delivery system and curcumin analogues are extensively reviewed. Therefore, nanoparticle curcumin complex could be clinically acceptable carrier for the regulation of telomerase activity of most of the cancer cells as well as development of novel therapeutic agents. Thus potentials of curcumin could be multifold enhanced and clinically significant.

(Received August 17, 2008; accepted August 20, 2008)

Keywords: Telomerase; Nanoparticles; Curcumin; Cancer drugs

1. Introduction

Curcumin a hydrophobic polyphenolic compound (molecular formula $C_{21}H_{20}O_6$) is the major biologically active and yellow phytochemical compound of Turmeric *Curcuma longa* (Zingiberaceae) [1]. Chemically, curcumin is a bis-α β-unsaturated β-diketone commonly known as diferuloylmethane, has recently gained a plenty of attention for its curative impact on a number of ailments [2-3]. Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural compound, widely used as a spice and coloring agent in food, possesses potent antioxidant, anti-inflammatory and anti-tumor promoting activities [3-4]. Commercially curcumin contains approximately 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin. Immunomodulatory effects of curcumin at low doses of curcumin have also been shown [5]. Although, curcumin is poorly absorbed after ingestion, multiple studies have suggested that even low concentrations of curcumin are sufficient for its chemopreventive and chemotherapeutic activity [5-6]. Recently, studies have shown that a chemopreventive effect of curcumin could be due to the hyper production of reactive oxygen species (ROS) inducing apoptosis in tumor cells. However, toxicologically, it is relatively inert and does not appear to be toxic to either animals [7] or humans [7] even at high doses. Curcumin has been shown to exhibit antioxidant, anti-

* Corresponding author: mishravijendra@yahoo.co.in.

inflammatory, antimicrobial and anticarcinogenic activities [8]. Furthermore, curcumin also shows the hepato- and nephro-protective, thrombosis supressing, myocardial infarction protective, hypoglycemic, and antirheumatic effects [8]. Thus, curcumin regulates multiple targets (multitargeted therapy), which is needed for treatment of most diseases, and it is inexpensive and has been found to be safe in human clinical trials. Various animal models [9-10] or human studies [11-12] proved that curcumin is extremely safe even at very high doses.

The absorption, biodistribution, metabolism, and elimination studies of curcumin have, unfortunately, shown only poor absorption, rapid metabolism, and elimination of curcumin as major reasons for poor bioavailability of this extremely human beneficial polyphenolic compound. Some of the possible ways to overcome these problems are discussed below. Nanoparticles encapsulated curcumin like nanocurcumin are one of the major means that could be used to improve its bioavailability due to its significant beneficial characteristics. Nano-crucumin complexes are the promising novel formulations, which appear to provide longer circulation, better permeability, and resistance to metabolic processes. Therefore the ability of curcumin indicates the possibility of developing as a potential universal cancer chemoprevention and chemotherapeutic agent. Thus, curcumin may regulate multitargeted human therapy, which is cost effective and also safe for human clinical trials.

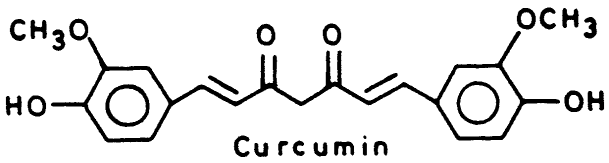

Fig. 1. The structure of curcumin

2. Role of curcumin in cancer antiproliferation

Literature shows that curcumin has potential as an antiproliferative, anti-invasive, chemopreventive and antiangiogenic agent as well as therapeutic agent in Alzheimer disease, Parkinson disease, cardiovascular disease, wound healing, diabetes, pulmonary disease, and arthritis [3, 5, 13]. Multiple research show curcumin regulates various immune cells like T lymphocytes (CD4), (CD8), B lymphocytes, natural killer cells, macrophages, dendritic cells and other immune cells [12]. Moreover, therapeutic potentials of curcumin has also been reported against various human diseases, including cancer, cardiovascular diseases, diabetes, arthritis, neurological diseases and Crohn's disease also [8]. *In vitro* studies have demonstrated curcumin's ability to promote apoptosis, among leukemia, B cell lymphoma and other cancerous cells [5]. Furthermore, it has been demonstrated that curcumin inhibits protein kinase C (PKC) [6], a kinase that plays a significant role in regulation of cytokine synthesis during growth of cancer cells [28]. Curcumin arrests the growth of cancer cells in the G2/S phases of cell cycle [14]. Curcumin also aids in control of tumor progression through its indirect actions and its ability to stimulate hosts' anticancer immune responses. Curcumin enhances immunity by stimulating the CD4+ T-helper cells and B lymphocyte [3]. Moreover, immunomodulatory potentials of curcumin has also been shown in modulating the activation of T cells, B cells, macrophages, neutrophils, natural killer cells and dendritic cells [15]. Evidences show curcumin`s potential to downregulate the expression of various pro-inflammatory cytokines including TNF, IL-1, IL-2, IL-6, IL-8 and IL-12 [16].

3. Telomere

Telomere is a repeating (5'-TTAGGG-3'), non-coding DNA sequence located at terminal ends of the chromosomes. Telomeres function by preventing chromosomes from losing base pair sequences at their ends [17]. However, each time a cell divides, some of the telomere length is lost (about 25-200 base pairs per cell division) [18]. As an outcome of this telomere becomes too short

and the chromosome reaches a "critical length" and can no longer replicate. Such cells are designated as senescent and are induced to undergo apoptosis [19]. Telomere is regulated by an enzyme namely telomerase, which is a ribonucleoprotein, with the function of a DNA polymerase, which synthesizes and adds the tandem hexameric telomeric repeats (TTAGGG) via reverse transcriptase reaction onto the 3' termini with substantial pausing after the addition of each hexamer, of existing telomeres using its one segment of RNA component as a internal template (natural primers for telomerase) [20]. Telomerease is mainly composed of an RNA template region (hTR); it provides a template r-5'-CUAACCCUAAC-3' [20] and a catalytic component the telomerase reverse transcriptase termed hTERT [21]. While analyzing human cancers, the positive frequency of human telomerase RNA component (hTR) and human telomerase reverse transcriptase (hTERT) was overwhelmingly displayed in cancers of the breast, colon, gallbladder, lung, stomach and oesophagus [22]. Telomerase, a reverse transcriptase, has been found to be activated in more than 80% of human cancers and, therefore, can be considered as a potential marker for tumorigenesis [23]. Telomerase is thus proving to be a reliable marker for the proliferating capacity and tumor mass of cancer patients [24]. Recent studies have shown that telomerase activity is found in 85% to 90% of all human cancers. Telomerase provides an exciting drug target for the prevention of cancer. This makes telomerase a good target not only for cancer diagnosis, but also for the development of novel therapeutic agents [25]. Activation of telomerase has been shown to be an important event for immortalization and carcinogenesis. Telomerase, playing a key role in making cancer cells immortal. Up-regulation of hTR expression has been shown to be an early event in various cancers [25]. The expression of *hTERT* seems to be coordinated with proliferation activity, because tumor cells possess high proliferative activity. Telomerase is not activated in the majority of normal somatic tissues suggesting the possibility that the *hTERT* gene product may be modulated during the process of translation and posttranslation in tumor cells [26].

Therefore, regulation of telomerase activity of in cancerous cells by nanocurcumin could be extremely beneficial to human beings. It was considered interesting to investigate actions of curcumin on signaling molecule activity. This review focuses on recent pharmacogenomic approaches to telomerase inhibition mediated by nanocurcumin. Drug design in the area of cancer therapeutics using nanoparticle could be developing a trend toward more precise mechanisms of cancer cell destruction.

4. Nanoparticles

Nanoparticles-based materials have attracted much attention in recent years because of their characteristic size and geometry dependent chemical and physical properties [27]. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Literature survey suggests nanoparticle research is an area of intense scientific research, due to wide potential applications in human therapy. Nano particles are sized between 1 and 100 nm. Nanoparticles have a very high surface area to volume ratio. This makes the particles very reactive or catalytic [27]. Nanoparticles are easier to pass through cell membranes in organisms and get interacted rapidly with biological systems [27].

5. Nanoparticle mediated curcumin delivery

Recently, nanoparticle technology emerged as a potential area of targeted drug delivery systems and make biologically availability of therapeutic agent. Nanoparticle-mediated delivery systems will probably be the most suitable for highly hydrophobic agents like curcumin, circumventing its poor aqueous solubility. However, very limited studies were made and the complete mechanism regarding nanoparticle mediated curcumin delivery system is still unknown. More recently a novel biosensor technique has been reported for determination of telomerase activity in cancer cells [28]. The detection of telomerase has proven very affective as a marker in tumors cells [28]. It has been found that about 80-90% of more than 950 primary tumors have been reported to express telomerase activity [28].

(i) Nanocurcumin

Recently, invention of nanocurcumin could be widely applied in the area of drug delivery for multitargeted therapy [3]. Moreover, Nanocurcumin, is a polymer based nanoparticle of curcumin, made up of the micellar aggregates of cross-linked and random copolymers of Nisopropylacrylamide (NIPAAM), with N-vinyl-2-pyrrolidone (VP) and poly (ethyleneglycol) monoacrylate (PEG-A). Nanoparticle encapsulated formulation of curcumin nanocurcumin [29, 31]. Nanocurcumin (size> 100nm) provides an opportunity to expand the clinical repertoire of this efficacious agent by enabling easier aqueous dispersion. Nanocurcumin, unlike free curcumin, is readily dispersed in aqueous media. Future studies utilizing nanocurcumin are warranted in pre-clinical in vivo models of cancer, downregulation of telomerase activity and other diseases that might benefit from the effects of curcumin. Literature survey suggests similar effect of nanocurcumin *in* V*itro* activity as that of free curcumin like in pancreatic cell lines [30-32], in down regulating the activation of transcription factor NFκB [32], induction of cellular apoptosis, down regulation of steady state levels of multiple proinflammatory cytokines (IL-6, IL-8, and TNFα) [32]. However, the potential beneficial activity over free curcumin *in* vivo effect is still unknown. Reports shows polymeric nanoparticle increase the stability of drugs and possess useful controlled release properties [8]. Furthermore, research nanoparticle especially solid lipid nanoparticles loaded curcuminoids (size 450nm) were shown to produce prolonged *in vitro* release of curcuminoids up to 12h for tropical applications [33]. Altogether, nanoparticle based curcumin delivery is still in its infancy and much progress is needed in this area.

(ii) Biobarcode assay

Recently, a novel telomerase assay (known as the biobarcode assay) method was developed using gold nanoparticle coated with short stretch of DNA mediated detection of telomerase activity [34]. Using biobarcode assay telomerase activity detected in 10 to 1,000 tumor cells *in vivo*.

(iii) Curcumin embedding phospholipid vesicles or lipid-nanospheres (Cm)

Recently a novel formulation to deliver curcumin embedding phospholipid vesicles or lipid-nanospheres (Cm) into tissue macrophages through intravenous injection has been developed. Moreover, Cm demonstrated easy solubilization in hydrophobic regions of these particles as well as nanoparticle dispersions also. In addition these formulations showed ability to scavenge reactive oxygen species as antioxidants in dispersions. Experimentally intravenous injection in rats showed massive distribution of Cm in cells assumed as macrophages into the bone marrow and spleen. Therefore lipid-based nanoparticulates could considered as improved intravenous delivery of Cm to tissues macrophages, specifically bone marrow and splenic macrophages and shown therapeutic potential as an antioxidant and anti-inflammatory [36].

(iv) Liposome curcumin for treatment of cancer

More recently, curcumin or a curcumin analogue encapsulated in a colloidal drug a liposome is considered as excellent drug delivery systems since they can carry both hydrophilic and hydrophobic molecules. Anticancer activity of liposome curcumin has been investigated *in vitro* and *in vivo*. Moreover, they show inhibition of human pancreatic carcinoma growth and exhibits antiangiogenic effect [37]. On the other hand growth inhibitory and apoptotic effects of liposomal curcumin has also been shown in colorectal cancer *in vitro* and *in vivo* [37].which might be due to the increased bioavailability of liposomal curcumin over free curcumin.

(v) Micelles

Experimental evidences shows 47% to 56% enhanced intestinal absorption of curcumin when embedded with micelles then free curcumin *in vitro* [38] in rats. On the other hand 60 fold

increase in biological half life for curcumin was observed when complexed with polymeric micellar, in rats as compared to curcumin solubilized in a mixture of DMA, PEG and dextrose [38]. On the other hand when curcumin phospholipid complex (100 mg/kg of curcumin) were administered orally male rats. About 1.5-fold increase in curcumin half-life in rats was demonstrated for the curcumin phospholipid complex over free curcumin. Therefore, curcumin phospholipid complex with curcumin could be advantageous for anticancer activity due to it's significantly delivery system [39].

(vi) Curcumin analogues

The molecular and chemical structure of curcumin plays a crucial role in its biological activity. Reports suggest change in antioxidant activity of curcumin due to isomerization [40]. With a view to achieve improved biological activity of curcumin through structural modifications or curcumin derivatives and/or its analogues research was made by various research groups [41]. For example, a curcumin analogue EF-24 had shown increased antitumor activity in comparison to curcumin *in vitro* and *in vivo,* and increased bioavailability of EF-24 was also demonstrated by 60% and 35%, respectively in male and female mice [42]. Various different curcumin analogues like symmetrical 1,5-diarylpentadienone were also shown 30 times tumor growth suppressive activity that of free curcumin. However, these analogues showed no *in vivo* toxicities [43].

(vi) Others

Another strategy to improve the biological activity of curcumin is by chelation with various metals as compared to free curcumin. The presence of two phenolic groups and one active methelene group in a curcumin molecule makes it an excellent ligand for any chelation. Moreover, improved antitumor activities of curcumin-copper complexes have also been reported as compared to free curcumin [44]. Furthermore, more than 10 fold increases in curcumin activity was demonstrated, when complexed with boron against HIV-1and HIV-2 proteases *in vitro* [45]. Further, the curcumin copper complex was equally effective as curcumin against cadmium induced oxidative damage in mice [50]. On the other hand higher reactive oxygen species scavenging ability was shown of curcumin and curcumin copper complexes than curcumin [40]. A vanadyl curcumin complex ($VO(cur)_2$) was reported to show a 2-fold increase in antirheumatic activity and a 4-fold increase in inhibiting smooth muscle cell proliferation as compared to free curcumin *in vitro*. Further, this complex was more effective as an anticancer agent, compared to uncomplexed curcumin [46].

6. Conclusions

However, the mechanisms underlying the anititumor abilities of nanoparticles are still not clear enough. The main strategies are to enhance in vitro and in vivo bioavailability of curcumin using nanocurcumin through structural modifications of the molecule and/or new formulations. However, the limited literature evidence devoted to show improvements in curcumin bioavailability reveals that the curcumin bioavailability enhancement has not gained significant attention. Yet, novel delivery strategies including nanoparticles, complexes offer significant promise and are worthy of further exploration in attempts to enhance the bioavailability, medicinal value. Nanocurcumin acts as a weapon to enter tumor cells and initiate their destruction which could emerge as an effective cancer-fighting agent.

Acknowledgements

The authors are thankful to Prof. (Dr.) Alok Jha, Institute of Agricultural Science, Banaras Hindu University is greatly acknowledged for fruitful discussion and suggestions.

References

[1] B.B.Aggarwal, A. Kumar, A.C.Bharti. Anticancer Res **23,** 363 (2003).
[2] R.J.Anto, A.Mukhopadhyay, K.Denning, B.B.Aggarwal. Carcinogenesis **23,** 143 (2002).
[3]A.Goel A, A.B.Kunnumakkara,B.B. Aggarwal. Biochem Pharmacol **75,** 787 (2008).

[4] C.A.C. Araÿjo, L.L Leon. Mem. Inst. Oswaldo Cruz. Rio de Janeiro **96**, 723 (2001).
[5] G.C.Jagetia, B.B.Aggarwal. J Clin Immunol **27,** 19 (2007).
[6] B.B.Aggarwal, C.Sundaram, N.Malani, H.Ichikawa. Adv Exp Med Biol **95,** 1 (2007).
[7] H. Ahsan, N. Parveen, N.U. Khan, S.M. Hadi. Chemico-Biological Interactions **121**, 161 (1999).
[8] P.Anand, A.Kumar, B.kunumarkkara, R.A.Newman, B.B.Aggrawal. Molecular Pharmaceutics **4**, 807 (2008).
[9] T.N. Shankar,N.V. Shantha,H.P. Ramesh, I.A. Murthy, V.S. Murthy. Indian J. Exp. Biol. **18,** 73 (1980).
[10] S. Qureshi, A.H.Shah, A.M. Ageel, Planta Med. **58**, 124 (1992).
[11] C.D.Lao, M.F. Demierre, V.K. Sondak. Anticancer Ther 6, 1559 (2006).
[12] G.Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran, P.S.Srinivas. Planta Med **64**, 353 (1998).
[13] A.C.Bharti, N.Donato,S. Singh, B.B.Aggarwal. Blood **101,** 1053 (2003).
[14] A. Khafif, S.P.Schantz, T.C.Chou. Carcinogenesis **19,** 419 (1998).
[15] Y.J. Surh, S.S.Han, Y.S.Keum. Biofactors **12,** 107 (2000).
[16] S.C.Gautam, X.Gao, S.Dulchavsky. Adv Exp Med Biol **95,** 321 (2007).
[17] R. K. Moyzis, J.M. Buckingham, L.S.Cram, M.Dani, L.L. Deaven, M.D.Jones, J.Meyne, R.L.Ratliff, J.R.Wu. PNAS USA **85**, 6622 (1998).
[18] M.Z. Levy, R.C.Allsopp, A.B.Futcher, C.W.Greider, C.B.Harley. J Mol Biol **225**, 951 (1992).
[19]S.P.Treon, N.Raje, K.C.Anderson. Semin Oncol.**27**, 598 (2000).
[20] J.Feng, W.S.Funk, S.S.Wang. Science **269**, 1236 (1995).
[21] T.M.Nakamura,G. Morin, K.B.Chapman. Science **277**, 955 (1997).
[22] J.W. Shay, Y.Zou, E.Hiyama, W. E. Wright. Human Molecular Genetics 10.677-685 (2001).
[23] N. Watanabe. Hokkaido Igaku Zasshi.**76**,127 (2001).
[24] R.K. Moyzis, J.M. Buckingham, L.S.Cram, M.Dani, L.L.Deaven, M.D.Jones, J. Meyne, R.L.Ratliff, J.R.Wu. Proc Natl Acad Sci. USA., **85**, 6622 (1988).
[25] G.X.Dong, C.Y.Rong. Chinese Medical Journal **120**, 755 (2007.
[26] N.W. Kim, M.A.Piatyszek, K.R.Prowse, C.B.Harley, M.D.West, P.L.Ho, G.M. Coviello, W.E.Wright, S.L.Weinrich, J.W.Shay. Science **266,** 2011 (1994).
[27] Upendra Kumar Parashar, P.S. Saxena, Anchal Srivastava. Digest Journal of Nanomaterials and Biostructures **3,** 81 (2008).
[28] E. Kulla, E. Katz. Sensors **8**, 347 (2008)
[29] R. H. Muller, C. M. Keck, J. Biotechnol. **113,** 151 (2004).
[30] D. Kirpotin, J. W. Park, K. Hong, S. Zalipsky, W. L. Li, P. Carter, C. C. Benz, D. Papahadjopoulos, Biochemistry **36,** 66 (1997).
[31] C. Karikar, A. Maitra, S. Bisht, G. Feldmann, S. Soni, R. Ravi. J. Nanobiotechnol **5,** 3 (2007).
[32] P. Anand, A. Kumar, B.Kunumarkkara, R.A.Newman, B.B.Aggrawal. Molecular Pharmaceutics **4,** 807 (2008).
[33] W. Tiyaboonchai, W. Tungpradit, P. Plianbangchang. Int. J. Pharm **337**, 299 (2007).
[34] G.Zheng, W.L. Daniel, C.A. Mirkin. J. Am. Chem. Soc., **130**, 9644 (2008).
[35] W. Tiyaboonchai, W. Tungpradit, P. Plianbangchang. Int. J. Pharm **337**, 299 (2007).
[36]K. Sou, S. Inenaga, S. Takeoka, E.Tsuchida. International journal of Pharm (2007).
[37] L. Li, B. Ahmed, K. Mehta, R. Kurzrock. Mol. Cancer Ther., **6**, 1276 (2007**)**.

[38] Z. Ma, A. Shayeganpour, D.R. Brocks, A.Lavasanifar, J. Samuel. Biomed. Chromatogr **21**, 546 (2007).

[39] A. Liu, H. Lou, L. Zhao, P. Fan, J. Pharm. Biomed. Anal **40**, 720 (2006).

[40] L. Shen, H.F. Ji, Acta A Mol. Biomol. Spectrosc **67,** 619 (2007).

[41] C.A. Mosley, D.C.Liotta, J.P. Snyder. Adv. Exp. Med. Biol. **595**, 77 (2007).

[42] A. Preetha, R. Banerjee, N. Huilgol. Cancer Invest **25**, 172 (2007).

[43] H. Ohori, H. Yamakoshi, M. Tomizawa, M.Shibuya,Y. Kakudo, A. Takahashi, S. Takahashi,S. Kato,T. Suzuki, C. Ishioka, Y.Iwabuchi, H.Shibata. Mol. Cancer Ther. **5**, 2563 (2006).

[44] V.D. John, G. Kuttan, K. Krishnankutty. J. Exp. Clin. Cancer Res **21**, 219(2002).

[45] Z. Sui, R. Salto, J. Li, C. Craik, P.R. Ortiz de Montellano. Bioorg. Med. Chem, **1**, 415 (1993).

[46] V. Eybl, D. Kotyzova, L. Leseticky, M. Bludovska, J.Koutensky. J. Appl. Toxicol. **26**, 207 (2006).

[47] K.H.Thompson, K. Bohmerle, E. Polishchuk, C. Martins, P. Toleikis, J.Tse, V.Yuen, J.H.McNeill, C. Orvig. J. Inorg. Biochem **98**, 2063 (2004).