Published in final edited form as:

Nutr Cancer. 2010 October ; 62(7): 883–890. doi:10.1080/01635581.2010.509537.

Nanochemoprevention: Sustained Release of Bioactive Food Components for Cancer Prevention

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Abstract

Chemoprevention, especially through the use of naturally occurring phytochemicals capable of impeding the process of carcinogenesis at one or more steps, is an ideal approach for cancer management. Despite accomplished outcomes in preclinical settings, its applicability to humans has met with limited success for many reasons including inefficient systemic delivery and bioavailability of promising chemopreventive agents. We have recently introduced a novel concept of “nanochemoprevention” that utilizes nanotechnology for enhancing the outcome of chemoprevention (*Cancer Res* 69, 1712–1716, 2009). To establish the usefulness of nanochemoprevention in cancer management, we studied the efficacy of a well identified chemopreventive agent epigallocatechin-3-gallate (EGCG) encapsulated in polylactic acid (PLA) and polyethylene glycol (PEG) nanoparticles (hereafter referred to as nano-EGCG) in preclinical settings. Nano-EGCG was found to retain its biological effectiveness, with over 10-fold dose advantage compared to nonencapsulated EGCG for exerting its cell growth inhibition, proapoptotic, and angiogenic inhibitory effects. Nano-EGCG was also observed to be effective in inhibiting tumor cell growth in athymic nude mice, with over 10-fold dose advantage compared to nonencapsulated EGCG. The rate of degradation of nonencapsulated EGCG was rapid, with a complete degradation within 4 h, whereas nano-EGCG had a significantly longer half-life. This study provides a foundation for the use of nanoparticle-mediated delivery of natural products to enhance the bioavailability of active agents for their enhanced effective and chemopreventive potential. In doing this, it is hoped that perceived toxicity concerns associated with prolonged use of agents could also be minimized. Oral consumption is the most desirable and acceptable form of delivery of chemopreventive agents. One disadvantage of using PLAPEG nanoparticles is its unstable nature in acidic environment; and therefore, it is not recommended for oral consumption. To overcome this obstacle, it will be important to develop nanoparticles encapsulating phytochemicals that are suitable for oral consumption.

CANCER CHEMOPREVENTION

Chemoprevention via the use of naturally occurring nontoxic agents has emerged as a plausible strategy for cancer management (1–4). *Chemoprevention*, by definition, is a means of cancer management by which the occurrence of the disease can be entirely prevented, slowed, or

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reversed via administration of one or more naturally occurring and/or synthetic compounds (2). The expanded definition of cancer chemoprevention also includes the therapy of precancerous lesions, which are called preinvasive neoplasia, dysplasia, or intraepithelial neoplasia, depending on the organ system. Among many diversified chemopreventive agents known for human cancer risk reduction, dietary phytochemicals are most practical and hold great potential. Most of the experimental studies conducted in the last 3 decades have suggested that plant derived products are the most acceptable and promising agents that could inhibit or delay various types of cancers (5). This information is supported by the fact that epidemiological studies suggest that consumption of fresh fruits and yellow-green vegetables reduce the cancer incidence and mortality due to stomach, colon, breast, lung, bladder, esophageal, prostate, and other cancers (5–7). Some of the well-identified chemopreventive agents, in addition to possessing preventive effects, are also showing therapeutic potential, and they often enhance the therapeutic efficacy of established chemotherapeutic agents. One such class of bioactive food components that has received considerable importance in experimental models of cancer is the polyphenolic group of compounds. Among all the polyphenolic compounds, green tea and its individual polyphenols have especially received considerable attention because of demonstrated efficacy against a variety of cancers (2,3,6,8).

It is important to mention that chemoprevention is distinct from the chemotherapy of established cancer. However, because of the expanded definition of chemoprevention, some degree of overlap between chemotherapy and chemoprevention exists. In addition, the term chemoprevention also includes prevention or inhibition of recurrence and development of new cancers in high-risk individuals. One misconception about chemoprevention is the thinking for complete prevention of cancer, an unachievable goal. Since the process of cancer development is carcinogenesis, we believe that our aim should be to prevent carcinogenesis, which in turn will lead to lower cancer burden. We, therefore, define chemoprevention as *slowing the process of carcinogenesis*, a goal that can be met.

STRATEGIES TO COMBAT CHALLENGES IN CHEMOPREVENTION

Many agents have been described to successfully retard carcinogenesis in several preclinical models. Despite these promising outcomes in preclinical settings, the applicability of chemoprevention to humans has met with only partial success. Few reasons for this limited success of chemoprevention in clinical trials are 1) diverse genetic background of individuals at risk; 2) varied food habits among the people: people eat food rather than a defined diet used in preclinical settings; and more important 3) inefficient systemic delivery; and 4) poor bioavailability of agents. Therefore, in order to achieve maximum response of a chemopreventive agent, novel strategies are required to enhance their sustained release for improved bioavailability of potentially useful agents. Such a strategy could in fact reduce the perceived toxicity associated with high doses that are typically required.

NANOCHEMOPREVENTION

Nanotechnology is an emerging interdisciplinary field that encompasses the disciplines of biology, engineering, chemistry, and medicine. Formal definitions of nanotechnological devices typically feature the requirements that “the device itself or its essential components be man-made, and be in the 1–100 nano meters range in at least one dimension” (9). It is noteworthy that in recent years, nanotechnology is being assessed and implemented in different areas of cancer therapeutics and cancer management and is expected to lead to major advances in cancer diagnosis, detection, and treatment (9–11). The basic rationale is that metals, semiconductors, and polymeric particles have novel optical, electronic, magnetic, and structural properties that are often not available from individual molecules and bulk solids (11,12). Because most biological processes, including those that are cancer related, occur at

nanoscale, nanoparticulate technology has been appreciated as a potential tool to diagnose and treat cancer. “Cancer nanotechnology,” as it is being referred to, is already being applied to cancer in two broad areas: the development of nanovectors, such as nanoparticles, which can be loaded with drugs or imaging agents and then targeted to tumors, and high throughput nanosensor devices for detecting the biological signatures of cancer. We recently employed the use of nanotechnology to improve the outcome of chemopreventive intervention and coined the term *nanochemoprevention* (13).

Nanoparticles in Cancer Chemoprevention

Nanotechnology at present is being utilized in cancer for molecular imaging, diagnosis, early detection, targeted therapy, and cancer bioinformatics. Cancer related nanodevices include but not limited to injectable nanovectors such as liposomes; biologically targeted, nanosized magnetic resonance imaging contrast agents; and novel, nanoparticles based methods (9). For delivery of a drug, the core of the nanoparticles can contain one or several payload drugs as well as permeation and visibility enhancers based on intended applications. The surface could also be bare or conjugated to targeting ligands such as polyethylene glycol, aptamer, or antibody to prevent macrophage uptake of the nanoparticles (14). Utilization of nanotechnology for the development of efficient anticancer drug delivery system is one of the most recent advancements in medical science. The structure and tunable surface functionality of a nanoparticulate system allows it to encapsulate/conjugate single or multiple entities either in the core or on the surface, rendering them ideal carriers for various anticancer drugs. Further, most drugs have poor solubility and low bioavailability and are formulated with undesirable solvents; and the use of nanocarriers allows for the preparation of low water soluble cancer medications as solid or liquid formulations. Nanoparticles made up of biodegradable and biocompatible polymers such as polylactic acid (PLA), poly (DL-lactide-co-glycolide acid; PLGA), starch, and chitosan, and so forth have been extensively utilized for the delivery of various drugs (14,15). Significant advantages of these biodegradable polymers are their history of safe use, proven biocompatibility, and ability to control the time and rate of polymer degradation and the release of the incorporated entity. Although several nanoparticles made up of biodegradable and biocompatible polymers have been studied for the delivery of various drugs, we used PLA nanoparticles because when these are injected systemically for drug delivery, they are rapidly cleared by endocytosis, thereby minimizing carrier induced undesirable cytotoxicity. In particular, homo and copolymers of lactic acid and polylactic glycolic acid have been extensively used for numerous drug deliveries (11,14,16). However, when PLA/PLGA nanoparticles are injected systemically for drug delivery, they are rapidly cleared by the mononuclear phagocyte system. Thus, the rapid removal of conventional nanoparticles, such as PLA/PLGA, from the blood stream limits their potential as controlled drug delivery vehicles (11). It is well known that the presence of a hydrophilic polymer such as polyethylene glycol (PEG) increases the circulation time of the PLGA nanoparticles by sterically stabilizing them against opsonization (14). This property of PEG thus improves pharmacokinetic and pharmacodynamic properties of the drugs that have been encapsulated in nanoparticles. PEGylation (i.e., the attachment of PEG to proteins and drugs) is an upcoming methodology for drug development, and it has potential to revolutionize medicine by drastically improving the pharmacokinetic and pharmacodynamic properties of administered drugs (17). Several investigators have demonstrated that PEGylated PLA/PLGA nanoparticles exhibit significantly increased blood circulation time and relatively lowered accumulation in different organs compared to non-PEGylated counterparts (18–20). Also, in vivo experiments have shown a significantly high accumulation of the PEGylated nanoformulation in the tumor tissues due to the enhanced permeation and retention effect (EPR) (Fig. 1). This EPR is mainly due to the difference in the vasculature between tumor tissue and normal tissue. Normal tissue vasculatures are lined by tight endothelial cells, which prevent the nanoparticles from escaping into the tissue; whereas tumor tissue vasculatures are hyperpermeable with leaky endothelium,

which easily allow the nanoparticles to infiltrate in the tissue. Once the nanoparticles carrying the drugs enter the blood flow, they move freely until they reach the tumor tissue where, due to the leaky environment, they allow the encapsulated drugs to be released and get accumulated in the tissue (Fig. 1 inset). The nanoparticles could also be conjugated with targeting moieties, which facilitate the nanoparticles to be delivered only to the tumor cells. The nanoparticles can be designed to encapsulate single or multiple agents and could also be conjugated with PEG to increase the circulation time of nanoparticles by stabilizing them against opsonization.

We recently envisioned that nanoparticle-mediated delivery could be implemented for sustained bioavailability of potentially useful chemopreventive agents. This sustained release and lower dose requirement could also limit perceived toxicity associated with their repeated use, a must for human use. To establish the proof of the principle of the concept, we assessed the effectiveness of delivery of a well-known chemopreventive agent, epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, encapsulated in PLA-PEG nanoparticles against human prostate cancer (PCa) under in vitro and in vivo situations (13). Our choice for the selection of EGCG and PCa in this study was based on following facts: 1) PCa is the most prevalent cancer among men, accounting for an estimated 192,280 new cases and 27,360 deaths in the year 2009 in United States alone, and because of long latency period, it is an ideal candidate disease for chemoprevention (21); 2) EGCG has shown remarkable chemopreventive potential in a wide range of cell culture studies (22,23); 3) our extensive experience with EGCG use in PCa; and 4) remarkable preclinical and clinical efficacy data of green tea and PCa (23–25).

Nanoencapsulated EGCG, Cell Proliferation, and Apoptosis

Our laboratory was the first to demonstrate that EGCG induces apoptosis in a variety of cancer cells both in vitro and in vivo, including cancer of the prostate gland (22,26–29). To establish the proof of the principle of nanochemoprevention, we tested the efficacy of EGCG encapsulated in PLA-PEG nanoparticles, hereafter referred to as nano-EGCG vs. nonencapsulated EGCG on proliferative ability of PCa cells. We observed that treatment of cells with PLA-PEG nanoparticles alone had an insignificant effect, whereas nano-EGCG, compared to nonencapsulated EGCG, produced remarkably superior effects at 24 h posttreatment, with over 10-fold dose advantage with an IC_{50} value of 3.74 μ M compared to 43.6 μ M of nonencapsulated EGCG. Similar effects of nano-EGCG were observed in 22Rv1 prostate carcinoma cell line (Fig. 2), indicating that the effects we observed are general and not cell-type specific. We also found that nanoencapsulation removes the penetration barriers at the PC3 cell surface and was readily available to the cells. We also observed that the effective concentration of EGCG was lowered by nanoformulation with 2.74 μ M nano-EGCG, resulting in similar extent of apoptosis as 40 μ M of nonencapsulated EGCG and thereby providing a remarkable dose advantage. Similar effects were also observed in 22R cells treated with nano-EGCG as compared to nonencapsulated EGCG. We found that 5.48 μ M nano-EGCG causes 81% apoptosis in 22Rv1 cell; whereas to achieve similar extent of apoptosis, over 40 μ M of nonencapsulated EGCG was required, thereby providing a remarkable dose advantage (Fig. 3). We also found that nano-EGCG provided a similar extent of dose advantage over nonencapsulated EGCG for inhibiting colony formation of prostate carcinoma PC3 cells.

This study also determined that EGCG retained its mechanistic identity when encapsulated in nanoparticles. The effects of nano-EGCG on several molecules, which have been extensively shown to be affected by EGCG, were determined; and the data demonstrated that the key regulators of apoptosis, Bcl-2 family of proteins, were significantly modulated by nano-EGCG. Like nonencapsulated EGCG, nano-EGCG treatment resulted in a significant increase in proapoptotic Bax levels with a concomitant decrease in the levels of antiapoptotic Bcl-2, thereby shifting the Bax/Bcl-2 ratio in favor of apoptosis. Further, an appreciable increase in

PARP cleavage as evident from cleaved PARP (85 kD fragment) was also observed. Importantly, these responses were observed at a very low concentration of nano-EGCG, further confirming a remarkable dose advantage when EGCG was delivered encapsulated in nanoparticles. This study also evaluated the effects of nano-EGCG on induction of the cyclin dependent kinase inhibitors WAF1/p21 and CIP1/p27 and observed a marked induction of p21 and p27 in a dose-dependent manner, with significant dose advantage over EGCG. Collectively, these data confirm that nano-EGCG retained its mechanistic signature and behaved exactly as EGCG with over 10-fold lower dose.

Nanoencapsulated EGCG and Angiogenesis

Earlier studies have demonstrated that EGCG is an efficient inhibitor of angiogenesis (30), which consecutively has been shown to have a direct association with the Gleason score, tumor stage, cancer progression and metastasis, and survival in patients with PCa (31). In our study, Ex ovo chick chorioallantoic membrane (CAM) was utilized for evaluating the effects of nano-EGCG on angiogenesis. We observed a 57% inhibition of FGF-induced angiogenesis with nano-EGCG containing only 3 µg EGCG compared to only 35% inhibition with 30 µg nonencapsulated EGCG. Further, this study also observed a significant inhibition of mean branch formation as well as tumor weight of neuroblastoma-induced angiogenesis in CAM by nano-EGCG, with a clear dose advantage over nonencapsulated EGCG. These data clearly indicate that although EGCG suppressed angiogenesis, the concentration required to achieve this inhibition was significantly reduced by nanoformulation.

In Vivo Efficacy of Nanoencapsulated EGCG

The relevance of in vitro responses always requires complementary in vivo validation. To determine the in vivo significance of our in vitro findings, we employed a xenograft mouse model. We compared the effect of nano-EGCG (100 µg/mouse; intraperitoneal administration) and nonencapsulated EGCG (1 mg/mouse; intraperitoneal administration) on the growth of tumors. The data demonstrated a significant decrease in the tumor volume at 45 days post-inoculation in both treatment groups as compared to control mice, with nano-EGCG demonstrating better efficacy than nonencapsulated EGCG. This data demonstrated that to achieve similar extent of tumor growth inhibition, a 10-fold lower dose of nano-EGCG was required compared to nonencapsulated EGCG. Further these data also demonstrated a significant inhibition in the serum prostate-specific antigen (PSA) of treated mice. The observed decrease of serum PSA by nano-EGCG at such a low concentration is an important observation because serum PSA is arguably regarded as the best marker in the diagnosis and prognosis of PCa in humans (32).

Limited bioavailability is supposedly the major obstacle believed to be responsible for the failure of naturally occurring chemopreventive agents in clinical settings. We found that the rate of degradation of nonencapsulated EGCG was rapid, with a complete degradation within 4 h. However, nano-EGCG had a significantly longer half-life. These data suggest nanoformulated EGCG given to mice is released slowly, which in turn may be responsible for its superior efficacy. This target specific enhanced bioavailability might be one reason for significantly better efficacy of nano-EGCG. Since EGCG has been demonstrated to act in synergy with other therapeutic approaches (33,34), we believe that nano-EGCG may offer a much better response when used in combination with other therapeutic approaches.

Oral Formulated Nanoencapsulated EGCG

Despite extraordinary advancement in other routes of drug administration, oral drug delivery remains well ahead an unavoidable and preferred delivery route. If it is a viable option, oral drug delivery is a preferred choice over other routes because of several factors including the ease of administration in nonhospital settings. However, with the use of PLA-PEG

nanoparticles oral delivery is not possible, as these nanoparticles are very unstable in acidic environment and thus could not be utilized for oral delivery. To overcome this obstacle, it will be important to formulate EGCG employing suitable nanomaterials such as chitosan.

CONCLUSIONS AND FUTURE PROSPECTIVE

Our proof of principle study (13) demonstrated the efficacy of nanoparticulate technology to enhance the therapeutic effectiveness of EGCG in vitro as well as in vivo. Our data clearly suggest that nano-EGCG exhibits over 10-fold dose advantage over nonencapsulated EGCG. We also demonstrated that EGCG retains its mechanistic identity upon nanoformulation that includes induction of apoptosis and inhibition of angiogenesis. We utilized PLA-PEG nanoparticles to provide EGCG to cancer cells, and the therapeutic/clinical significance of PLA-PEG nanoparticles remains in the fact that they rarely pose any toxicity against cells (35). Moreover, being biodegradable, these nanoparticles are perceived to be safe in the biological system (14). It will be important to resolve the problem of oral consumption of nanoencapsulated EGCG by incorporating biodegradable polymers as the starting material, which will be more stable in acidic environment of the gut to slowly release the EGCG for absorption by the body.

In addition to our study, a limited number of studies from other laboratories have also evaluated the usefulness of nanotechnology for delivery of natural products. In a recent study, poly (lactide-co-epsilon-caprolactone) was successfully developed as an EGCG-eluting polymeric stent that could be utilized for preventing thrombosis, inflammation, and in-stent restenosis (36). Utilization of nanotechnology for delivery of natural products is not limited to EGCG only. Recent studies (37,38) have demonstrated that curcumin could also be delivered via nanotechnology-based carriers for prevention and therapy of cancer. Bioactive food components for which nanotechnology has been exploited are summarized under Table 1. These and other studies on the subject have suggested to us that nanotechnology-based delivery may have considerable advantage over currently employed therapies for cancer. Nanochemoprevention (i.e., delivery of bioactive food components via nanotechnology-based carriers) could be developed as an inexpensive, tolerable, and readily pertinent approach for cancer control and management. This advancement might help us achieve the supraphysiological concentrations of the phytochemicals that are unachievable when the agents are administered as part of the diet. With current studies in mind, our ultimate goal could be once a day or once a week capsule containing one or more nanoencapsulated phytochemical (s) for effective chemoprevention and chemotherapy of cancers of target organs.

Based on our promising data and studies from other laboratories, we suggest that the concept of nanochemoprevention for cancer prevention should be explored further for its potential use in preclinical, phytochemical-based, chemopreventive studies. Supportive data from these studies could pave the way for effective chemoprevention of cancer in the human population.

Acknowledgments

This work was supported by U.S. PHS Grants RO1 CA 78809, RO1 CA 101039, and RO1 CA 120451. I. A. Siddiqui was supported by a postdoctoral fellowship by U.S. PHS Grant T32AR055893.

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FIG. 1.

Tumor targeting strategy with nanoparticles. Normal tissue vasculature is lined by tight endothelial cells, which prevents the nanoparticles from escaping into the tissue; whereas tumor tissue vasculature is hyperpermeable with leaky endothelium, which easily allow the nanoparticles to infiltrate and extravasate to the tissue. Once the nanoparticles carrying the phytochemicals enter the blood flow, they move freely until they reach the tumor tissue where due to the leaky environment, they allow the encapsulated drugs to be released and get accumulated in the tissue. The nanoparticles could be conjugated with targeting moieties, which will facilitate the nanoparticles to be delivered only to the tumor cells. The nanoparticles can be designed to encapsulate single or multiple agents and direct antitumor action. Inset: Enlarged tumor endothelium to demonstrate the passage of drugs encapsulated in nanoparticles. The nanoparticles carrying the phytochemicals are shown with targeting moieties that help them to bind on the tumor tissue and then release the encapsulated phytochemical(s) into the tissue. The nanoparticles could also be conjugated with polyethylene glycol (PEG) to increase the circulation time by stabilizing them against opsonization. PLA, polylactic acid.

FIG. 2.

Comparative effects of nonencapsulated epigallocatechin-3-gallate (EGCG) and nano-EGCG treatment on cell viability. A: 24 h cell growth analysis and B: 48 h cell growth analysis. 22Rv1 cells were treated with EGCG, void-nanoparticles, and nano-EGCG for specified time, and cell growth was determined by MTT assay in a 96-well plate. Lines are mean ($\pm$ SE) of 3 separate experiments in which each treatment was repeated in 10 wells. IC₅₀, inhibitory concentration of 50%.

FIG. 3.

Comparative effects of nonencapsulated EGCG) and nano-EGCG treatment on apoptosis. Quantitative estimation of apoptosis: 22Rv1 cells were treated with agents for 48 h, labeled with 2'-deoxyuridine 5'-triphosphate using terminal deoxynucleotide transferase and propidium iodide by using an Apo-direct apoptosis kit. The values shown inside each box indicates extent of apoptosis. The images are representative of 3 independent experiments with similar results.

TABLE 1

Studies employing nanotechnology for delivery of bioactive food components^a

Natural Agent	Nanoparticles	Target Organ	Outcome	References
EGCG	PLA-PEG	Prostate cancer	Enhanced bioavailability and limited unwanted toxicity	(13)
	PLCL	Vascular smooth muscle cells	Fabrication of EGCG-eluting vascular stent	(36,39)
	PLGA-DMAB	Rat model of cyclosporine (CyA)-induced chronic nephrotoxicity	Improved therapeutic efficacy	(36,39)
Curcumin	Alginate-Chitosan-Pluronic	HeLa cells	Cellular internalization of the agent	(40)
	mPEG-PA	Lypase and Hela cell lysates	Encapsulation of hydrophobic compound, good stability in physiological condition	(37)
	Liposomes	Prostate cancer	Significant dose advantage	(38)
	Poly(ϵ -caprolactone)	Diabetic wound dressing	Reduced inflammatory induction, increased rate of wound closure	(41)
	PEG	Pancreatic cancer	More potent effects on cell growth inhibition than free agent	(42)
Resveratrol	Liposomes	Head and neck squamous cell carcinoma	Suppressed HNSCC growth in vitro and in vivo, a viable nontoxic therapeutic agent	(43)
	mPEG-PCL	Glioma cells	Significant dose advantage	(44)
	Liposomes	Human embryonic kidney	Prevented the cytotoxicity at high concentrations	(45)
	Polymeric micelles	Rat adrenal medulla PC12 cells	Protected cells from Abeta-induced damage	(46)
	Emulsion and liposomes	Rat carotid artery	Enhanced preventative and therapeutic benefit	(47)
Curcumin and resveratrol	Liposomes	Prostate cancer	Enhanced serum levels, better efficacy in combination of agents	(48)
EGCG, tannic acid, curcumin	Gelatin	Breast cancer	Retained biological activity and blocked HGF-induced intracellular signaling	(49)

^a Abbreviations are as follows: EGCG, epigallocatechin-3-gallate; PLA, polylactic acid; PEG, polyethylene glycol; PLGA, poly (DL-lactide-co-glycolide acid; DMAB, p-dimethylaminobenzaldehyde; mPEG-PA, methoxy PEG palmitic acid; HNSCC, head and neck squamous cell carcinoma; mPEG-PCL, methoxy PEG poly(ϵ -caprolactone).