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Bioavailability of phytochemicals and its enhancement by drug delivery systems

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Abstract

Issues of poor oral bioavailability of cancer chemopreventives have hindered progress in cancer prevention. Novel delivery systems that modulate the pharmacokinetics of existing drugs, such as nanoparticles, cyclodextrins, niosomes, liposomes and implants, could be used to enhance the delivery of chemopreventive agents to target sites. The development of new approaches in prevention and treatment of cancer could encompass new delivery systems for approved and newly investigated compounds. In this review, we discuss some of the delivery approaches that have already made an impact by either delivering a drug to target tissue or increasing its bioavailability by many fold.

Keywords

Chemoprevention; Bioavailability; Nanochemoprevention; Drug delivery; Polymeric implant; Nanoparticles

1. Introduction

Since ancient times, plant-derived compounds have been a great source of materials used in beneficial medical treatments. Many plant extracts have been tested in numerous systems to assess their chemopreventive and chemotherapeutic efficacy. Since the advent of advanced chromatography systems to isolate substantial amounts of compounds from complex mixtures and the advancement of chemical synthesis methods, several isolated phytochemicals and synthetic chemicals/metabolites have been tested *in vitro* and *in vivo*. Many compounds identified as promising agents have been successfully translated to marketable drugs [1]. As the cancer prevention field has developed, many researchers have turned to plants as a source for identifying new potent agents.

Dietary supplements have gained traction for use in chemoprevention, based on multiple epidemiological studies correlating higher intake of fruits and vegetables with lower cancer risk [2– 4]. Based on the conclusions drawn from such studies, several long-term randomized controlled trials of the isolated compounds of plant origins have been initiated.

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However, many of those trials were unsuccessful in providing convincing experimental evidence, or they were abandoned due to adverse effects, including increased risk of the cancer for which they were tested. Multiple studies with β-carotene, vitamin A, vitamin C, and α-tocopherol, either individually or in combination, showed no evidence for protection against cancer incidence or mortality. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) in prostate cancer was halted following a non-significant increase in prostate cancer incidence and that it was unlikely to achieve clinical benefit [5]. Furthermore, a follow- up study of the subjects revealed a significant increase in prostate cancer incidence years later in the high vitamin E group, though the subjects were no longer taking the supplements [6]. The Southwest Oncology Group (SWOG) trial of selenium against prostate cancer also showed no benefit [7]. The Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) trial in France, testing a combination of vitamin C, vitamin E, β-carotene, selenium, and zinc, also failed to show any overall benefit in cancer incidence [8]. Apart from these null findings, the β-carotene and Retinol Efficacy Trial (CARET) reported a 39% increase of lung cancer compared with the placebo. In the α-Tocopherol, β-carotene Cancer Prevention Trial (ATBC), a 16% increase in lung cancer was found to be associated with treatment [9]. All these trials tested plant products that showed powerful antioxidant properties in preclinical studies. Based on the hypothesis that antioxidants would decrease the oxidative stress induced by the process of carcinogenesis, it was assumed they would be effective in cancer prevention, and hence the trials had originally been established.

Although these studies do not definitively prove that antioxidants have no anticancer effect, they do raise several possible explanations of the contradictory preclinical versus clinical trial results. For example, it is possible that one compound has been falsely identified as the bioactive principle in a system where multiple agents and interactions between multiple regulatory molecules could be responsible for the biological effect of interest. Meyskens and Szabo have postulated that most of the epidemiological studies identify one micronutrient as causative factor, while the levels of other micronutrients or biological parameters are not taken into account [10]. They highlight the view that micronutrients or other dietary components do not act in isolated form, but rather results of epidemiological studies on dietary factors are due to the entire dietary package. Many epidemiological studies also look for surrogate end points of benefit, often ignoring the long-term, good or harmful outcomes [11]. Dietary questionnaires in epidemiologic studies may sometimes result in misleading conclusions, where cases and controls are compared for effects of supplements alone, and this can often lead to inverse associations [12]. Thus, looking at effects of one isolated antioxidant might not result in the same effects as testing the total diet. Phytochemical antioxidants are also essential micronutrients that play a significant role in normal cellular metabolic processes. Dietary supplements that increase the biological levels of essential micronutrients may also have a deleterious effect on normal metabolism [11]. Studies focusing on non-essential, non-nutritional antioxidants might provide an alternative approach to studying effects of antioxidants. Furthermore, actively preventing/intercepting cancer using risk-reducing agents, termed ‘cancer interception’, has proven challenging, mostly due to lack of measurable factors [13].

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Epidemiological studies on the dietary and lifestyle habits influencing the outcome of cancer incidence has led to screening of several plant-based products in laboratory settings. From such work, multiple compounds that display potential anticancer properties *in vitro* were identified [14,15]. However, when those compounds were taken into *in vivo* studies, many of them failed to translate the preclinical findings. It was found that the compounds were unstable in the gut and exhibit poor bioavailability, which likely could be reasons for the clinical failure. To overcome poor bioavailability, higher doses were tested, which showed efficacy but resulted in toxicity in several organs [16].

Bioavailability of a compound cannot be accurately predicted; however, analysis by Lipinski's 'rule of five' provides some insight: in general, a compound will have better bioavailability when it contains not more than 5 hydrogen-bond donors, not more than 10 hydrogen-bond acceptors, has a molecular mass not greater than 500 daltons, a partition co-efficient log P-value of not greater than 5 and contains less than 10 rotatable bonds [17]. Most of the chemopreventive agents, including polyphenols such as curcumin and green tea polyphenols, do not fall within these specifications, and they exhibit low bioavailability [18]. However, compounds such as genistein and biochanin A, which, according to the rule, should have good absorptive properties, are excreted by an efflux mechanism into the gut at a high rate that limits bioavailability [18]. Thus, several other factors could also play a role in limiting bioavailability, such as solubility of the compound, stability due to gastric and colonic pH, metabolism by gut microflora, absorption across the intestinal wall, active efflux mechanism and first-pass metabolic effects. Examples of compounds that are only sparingly water-soluble include ellagic acid, curcumin and resveratrol [19,20], while bioavailability of epigallocatechin gallate is limited by poor absorption and rapid first-pass metabolism [21]. For many chemopreventives, although only a small fraction of the compound is absorbed/metabolized by the intestinal epithelium, that tissue nonetheless plays a major role in bioavailability through the uptake and efflux transporters that are present on the epithelial cell surface. The major transporters that are relevant to chemopreventive agents are P-glycoprotein, breast cancer resistance protein and multidrug resistance protein 2 [22]. The efflux transporter proteins belonging to the ATP-binding cassette gene family mediate the active extrusion of many nutrients, drugs, and metabolites back into the intestinal lumen [23]. Thus, even when higher concentrations of the chemopreventive agents are present in the intestinal lumen, only nM concentrations were available in the blood [24]. Therefore, it may be difficult to assess bioavailability of agents based solely on their physicochemical properties.

The bioavailability issue of the compound of interest at the target site is of highest relevance, but curiously it has received the lowest priority in the field of cancer prevention [25]. The detectable plasma level of a compound does not directly address its bioavailability, since the bioaccumulation of the compound at the target site is the critical factor relating to its efficacy. If compounds tested in cell culture models fail to achieve effective concentration in the target milieu *in vivo*, even when they have been shown to modulate key metabolic pathways involved in tumorigenesis, then results of the *in vitro* studies become irrelevant. Thus, after pilot *in vitro* studies demonstrating the efficacy of a compound and

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before detailed mechanistic studies are undertaken, compound bioavailability at the organ of interest needs to be established.

Understanding the issues related to bioavailability of individual compounds could lead to approaches to bypass their limitations. Many studies have concluded that rapid conjugation of a compound, especially by glucuronidation in the intestine and liver, is primarily responsible for poor bioavailability [25]. Glucuronidation is mediated by uridine diphosphate-glucuronosyltransferases (UDP-UGTs) [26], and together with reactions of cytochrome P450 enzymes, these represent more than 80% of the pathways involving compound metabolism. Glucuronidation and cytochrome P450 pathways are recognized as important clearance mechanisms [27]. One approach to increase the bioavailability of chemopreventives could be co-delivery of agents that modulate activity of such pathways or, in a broader sense, inhibit the metabolism of the tested compound *in vivo*.

It has been shown that piperine, a component of black pepper (*Piper* spp.), inhibits glucuronidation of several chemopreventive compounds, thus enhancing their bioavailability by modulating enzymatic drug biotransforming reactions *in vitro* and *in vivo* [28,29]. Piperine was shown to reduce the glucuronidation rate *in vitro* and also to inhibit arylhydrocarbon hydroxylation, 7-ethoxycoumarin-O-deethylation and ethyl morphine-N-demethylation. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and UDP-UGT. It has also been shown to increase the bioavailability of a number of therapeutic drugs as well as phytochemicals, including tea polyphenol epigallocatechin-3-gallate, in mice [30]. Piperine's bioavailability-enhancing property may also be attributed partly to increased intestinal absorption because of its effect on the ultrastructure of intestinal brush border [31]. Ultrastructural studies revealed that piperine increases microvilli length, increase in intestinal brush border membrane fluidity thus altering membrane dynamics and permeation characteristics that result in increased small intestinal absorptive surface [32]. Similarly, curcumin is absorbed in the intestine but is rapidly metabolized in both intestinal epithelial tissue and liver. Thus, very little curcumin reaches the circulation intact for subsequent bioaccumulation at the target organ [33,34]. Addition of piperine along with curcumin treatment increases the circulating curcumin plasma levels by inhibiting the glucuronidation of curcumin and thereby inhibiting its elimination from the circulation [35,36].

Resveratrol, the main active polyphenol in red wine, has been shown to have cancer chemoprevention potential, but its bioavailability is limited due to various reasons, including low stability, increased oxidation, low solubility and high hepatic uptake [37]. Data suggest that during metabolism, the majority of resveratrol undergoes glucuronide conjugation catalyzed by UGTs (resulting in 3- and 4-O-glucuronides), and sulfate conjugation by sulfotransferases [38]. Thus, effects on resveratrol stability *in vivo* could be studied in combination with inhibitors of UGTs and sulfotransferases. Quercetin and myricetin have been shown to inhibit glucuronidation and sulfation of resveratrol, and thereby to increase resveratrol bioavailability [39,40]. However, these results contradict those of a study from another group [41], who found no difference in effects obtained with resveratrol alone or in combination with quercetin. However, the combined effects may have been underestimated in that study since only trans-resveratrol levels were measured.

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A caveat to this approach is the long-term effect on metabolism of xenobiotics, which when hindered, can lead to other complications, including cancer. Therefore, modulating compound metabolism to increase bioavailability is not a viable option for long-term cancer prevention. Rather, a better compound delivery system to achieve efficacious levels at the target organ is needed.

2. Drug delivery systems to increase bioavailability

Clinical medicine possesses an abundance of pharmaceutical products for therapeutic use, and the list is increasing rapidly with greater understanding of molecular mechanisms of diseases. However, favorable drug action alone against the disease is insufficient to satisfy the medical community; in addition, avoiding undesirable drug actions on normal tissues, as well as minimizing side effects of the therapy, is equally important. Clinically, the therapeutic efficacy of an anticancer drug relies not only on its intrinsic anticancer activity but also on the bioavailability of the drug at the target site.

Many agents have low aqueous solubility, and this is associated, in general, with low oral bioavailability [42]. In the development of novel therapeutics, the ability to devise a suitable pharmaceutical formulation for delivery is of utmost importance. Therefore, the means to deliver chemopreventives are critical for effective prevention and treatment of cancer. The emergence of new technologies has engendered great interest in developing novel drug delivery systems to advance both the pharmacological and therapeutic properties of parenterally administered drugs.

A promising approach to overcome low bioavailability and systemic toxicity is the application of drug-loaded nanosized drug carriers, such as polymeric nanoparticles (NPs), liposomes, dendrimers and micelles [43,44]. Use of such carriers has several advantages compared to systemic chemotherapy, it can modulate the pharmacokinetics of existing drugs, and it may be useful to enhance delivery of anticancer agents to target sites. In this review, we discuss some of the delivery methods that have already made an impact either by enhancing delivery of the drug to its target tissue or increasing its bioavailability by many fold.

2.1. Nanoparticles

There has been considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. NPs range in size from 10 to 1000 nm and can be synthesized from lipids, proteins and carbohydrates, as well as several natural and synthetic polymers. For delivery, a drug is dissolved, entrapped, encapsulated or attached to an NP matrix. Depending upon the method of preparation, NP, nanospheres or nanocapsules can be obtained. NP systems are being explored for a variety of biomedical applications. Their use to improve the therapeutic index of encapsulated drugs either by protecting them from enzymatic degradation [45], altering pharmacokinetics [46], reducing toxicity [47] or providing controlled release over extended periods of time [48] has gained enormous acceptance of NP systems in the last decade, as reviewed recently [42].

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NPs may enhance the oral bioavailability of poorly soluble drugs and the tissue uptake after parenteral administration, through adherence to the capillary wall. They also enhance the delivery of certain drugs across membranes. Being small in size, NP have the potential to leave the vascular system and enter sites of inflammation [49]. The NP size limitation for crossing different biological barriers is dependent on the tissue, target site and circulation [50]. NPs are subject to phagocytosis and endocytosis. Due to their hydrophobic surface, they are rapidly opsonized (coated) by plasma proteins and taken up by the mononuclear phagocytic system (MPS), which is found in organs such as liver, spleen and bone marrow. However, coating with polyethylene glycol (PEG) or hydrophilic copolymers results in increased hydrophilicity, which allows prolonged circulation in the bloodstream and thus potentially enhanced uptake in non-MPS organs and accumulation at sites of inflammation [49].

There are several kinds of NP used in drug delivery systems, and some are discussed in this article under different headings. One kind is the self-assembled NP. Self-assembled nanocarriers are generally characterized by a hydrophobic core and hydrophilic shell, are considered as superior drug carriers and have been developed by several research groups [51,52]. A second kind of NP is the polymeric NP, which may be synthesized by various methods [53], according to needs of the application and type of drug being encapsulated. Polymeric NPs have properties of controlled/sustained release, subcellular size and biocompatibility with tissue and cells [54]. Solubility and pharmacokinetic properties of drugs may be improved by encapsulation within NPs. This delivery approach could enable further clinical development of chemical entities that have stalled because of poor pharmacokinetic properties [55]. Several researchers have used different types of NP for chemoprevention by narigenin [56], curcumin [57] and epigallocatechin gallate [58,59]. Nanomedicines are stable in blood, non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory, do not activate neutrophils, are biodegradable and applicable to delivery of various types of molecules, such as drugs, proteins, peptides or nucleic acids [60].

Stimuli-responsive polymer-based NPs have received much attention in areas of drug and gene delivery, tissue engineering and biosensors [61,62]. Such NPs undergo abrupt physical or chemical change in response to change of environmental conditions, such as pH, temperature, light, magnetic field or glucose [63,64]. Colson and Grinstaff [65] have recently reviewed biologically responsive polymeric NPs for drug delivery that release their drug cargo in response to a change in pH or oxidative stress. These would be of significant clinical interest as they offer the opportunity to link drug delivery to a specific location or disease state. One example is paclitaxel (PAC) delivery by loading on pH-responsive NPs. This system has been tested against MDA-MB-231 human breast cancer cells *in vitro* and demonstrates superior cytotoxicity when compared to PAC delivery on non-responsive polycaprolactone (PCL) NPs [66]. In another study, PAC delivery on pH-responsive NPs demonstrated greater efficacy *in vivo* against subcutaneous SKOV-3 tumors compared to free PAC [67].

There is almost undivided opinion among researchers in the field that the utilization of the full potential of nanotechnology requires attention to safety issues. There is little experimental toxicity data available on the vast range of NPs. However, its long-term use

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could lead to potential risk for toxicity. One of the primary mechanisms of nanoparticle toxicity is production of ROS and free radical due to foreign body reaction leading to oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA [68,69]. NP-induced oxidative stress occurs during the dissolution of iron-based NPs, which catalyzes ROS generation and formation of $OOH^{\cdot}$ and $OH^{\cdot}$ radicals from H_2O_2 via the Fenton reaction [69,70]. Moreover, some studies suggest that NPs are not inherently benign and that they affect biological behaviors at the cellular, subcellular, and protein levels [71,72]. Although inspiring from biological viewpoint, polymeric NPs has also been reported to trigger detrimental responses. Nanopolymers made of silica dioxide have been shown to increase the kidney weight and creatinine levels when given intraperitoneally at 200 mg/kg body weight in *in vivo* animal model [73]. Another potential challenge for biodegradable polymeric nanoparticles is associated with solvent residues and polymer toxicity. NPs prepared from different materials like copper [74], silica [75], TiO_2 [76], gold [77], silver [78] and polystyrene [79] have shown potential toxicity in murine models when delivered oral or intravenous. However, NPs formed from biodegradable materials such as PLGA [80] and PCL [81] are expected to demonstrate none or fewer toxic events than non-biodegradable materials (reviewed in [69,70]).

2.2. Liposomes

Liposomes are nanosize artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol. These vesicles have been reported to serve as immunological adjuvants and drug carriers [82,83]. Though liposomes can vary in size from nanometers to tens of micrometers, generally ranging from 25 nm to 2.5 μm [84]. The distinct advantages of liposomes are their ability to encapsulate various materials and their structural versatility. Liposomes can encapsulate drugs with widely varying solubility or lipophilicity, either entrapped in the aqueous core of the phospholipid bilayer or at the bilayer interface [84].

Liposomes composed of natural lipids are biodegradable, biologically inactive, weakly immunogenic [85], produce no antigenic or pyrogenic reactions and possess limited intrinsic toxicity [86]. Therefore, drugs encapsulated in liposomes are expected to be transported without rapid degradation and to result in minimum side effects for the recipients.

Liposomes are increasingly used by the pharmaceutical industry to deliver certain drugs, vaccines and enzymes for the prevention or treatment of a variety of diseases. Liposomes have been investigated for delivery of chemotherapeutic agents for cancer treatment [84,87], vaccines for immunological protection [88], radiopharmaceuticals for diagnostic imaging [89] and nucleic acid–based medicines for gene therapy [90]. Several formulations have been developed and studied with regard to relative stability, pharmacokinetic properties, biodistribution and toxicity. The compounds entrapped into liposomes are protected from the action of external modulatory factors, particularly enzymes and inhibitors [91]. Moreover, liposomes are able to deliver drugs into cells by fusion or endocytosis, and practically any drug, irrespective of its solubility, can be entrapped into liposomes.

Despite the many advantages of liposomes, including safety and biocompatibility, their main drawback as nanocarriers is their instability in plasma [92]. On intravenous liposome

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administration, selective serum proteins (opsonins) bind to their surface, thus signaling their presence. After signaling, the liposomes are then rapidly captured by the MPS and removed from the blood circulation. Interestingly, this very behavior has been exploited for efficient delivery of antiparasitic and antimicrobial drugs to treat infections localized in the MPS [93,94]. However, when the target site is beyond the MPS, the use of liposomes that are able to evade this system is required to reach longer circulation times.

Reports suggest that prolonged circulation time of liposomes may result in significant accumulation in highly vascularized, permeable tissues such as tumors [95], especially in cases involving active neoangiogenesis. Tumor localization of long-circulating liposomes, such as PEG-coated (pegylated) liposomes, has a passive targeting effect that may enable substantial accumulation of encapsulated drug in interstitial fluid at the tumor site [96]. Based on this rationale, pegylated liposomal doxorubicin delivery for cancer therapy was achieved. In this formulation, PEG coating protected the liposomes from opsonization and recognition by the reticulo-endothelial system, which resulted in prolonged circulation time, and enhanced accumulation in tumors [97]. Preclinical experiments indicate that stealth liposomal delivery of anthracyclines decreases the cardiotoxic effect, enhances antitumor activity, and improves the overall therapeutic index [98].

2.3. Micelles

Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions. Polymeric micelles range from 10 to 100 nm in size, and they are usually very narrow [51]. The critical association concentration of polymer is lower by several orders of magnitude than typical critical concentration values for surfactant micelles, which makes polymeric micelles more stable toward dilution in biological fluids. They can increase drug bioavailability and retention, since the drug is well protected from possible inactivation by its micellar surroundings [99]. Drug release from micelles is governed by various factors, such as micelle stability, rate of drug diffusion, the partition coefficient and the rate of copolymer biodegradation [100]. The drug concentration within the micelles, the molecular weight, physicochemical characteristics of the drug and its location within the micelles can also affect drug release [101]. As discussed for NPs, drug release from the appropriate types of micelles can also be enhanced in the targeted area by certain physical stimuli, such as pH, temperature, ultrasound and light [102].

In the last decade, polymeric micelles designed from amphiphilic block copolymers have been found to hold a significant potential as drug delivery vehicles for a variety of anticancer drugs due to unique properties, such as high solubility and low toxicity [103]. Apart from improving drug solubilization, small particle size, long circulation, targeting and easy production properties, polymeric micelle systems can alter the drug internalization route and subcellular localization. They can also lessen the P-glycoprotein efflux effect and, consequently, exert a different mechanism of action from the entrapped drugs [104]. They also have physicochemical properties for tumor targeting by an enhanced permeability and retention effect that is a type of passive targeting mechanism, leading to a higher drug concentration at the tumor site and decreased side effects compared with systemic administration [105]. Furthermore, compared with more recent nanodrug delivery systems,

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including liposomes, NPs and dendrimers, polymeric micelles possesses higher drug-loading capacity as well as improved stability [106].

The polymeric micelle system, owing to its solubilization, selective targeting, P-glycoprotein inhibition and subcellular localization properties, has received growing scientific attention as an effective drug carrier. The micelle delivery system has been widely accepted, and currently seven different polymeric micelle formulations of antitumor drugs are being evaluated in clinical trials [106]. Hydrophobic drugs, in general, can only be administered intravenously (i.v.) after addition of solubilizing adjuvants like cremophor EL or ethanol, and this is often accompanied by toxic side effects [107]. Incorporation of such drugs in nanocarrier-like micelles avoids the use of adjuvants [108].

The high toxicity of potent chemotherapeutic drugs like PAC, doxorubicin (DOX) and many others limit the therapeutic window in which they can be applied. This window can be expanded by controlling the drug delivery in both space and time such that non-targeted tissues are not adversely affected. Xiao and co-workers [109] have recently described a method for DOX delivery by covalent conjugation to the hydrophobic segments of amphiphilic block copolymer arms via a pH-labile hydrazone linkage, which enables pH-controlled drug release. The unimolecular micelles exhibited a uniform size distribution and pH-sensitive drug release behavior. Similarly, it was shown that rapamycin could be loaded efficiently in mixed micelles up to a concentration of 1.8 mg/mL by a hot-shock protocol. The release kinetic studies of rapamycin demonstrate that this type of micellar system could be triggered by varied pH environments under physiological conditions [110]. In another study, Matsumura and colleagues demonstrated that a PAC micellar formulation consisting of PEG and modified polyaspartate as hydrophobic block showed a similar cytotoxicity in 12 human tumor cell lines (lung, gastric, esophagus, colon, breast and ovarian) compared to PAC alone [111].When tested in preclinical studies *in vivo* in colon 26-bearing CDF1 mice, an over 50- times higher area under the curve was achieved, while the maximum plasma concentration (C_{max}) in tumors was 3-times higher compared to PAC alone [112]. In summary, polymeric micelle systems have become increasingly important in oncology, and so far the evidence points to an increasing hope for use in cancer therapy.

2.4. Niosomes

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol, with subsequent hydration in aqueous media [113]. They resemble liposomes in their architecture and can be used as an effective alternative to liposomal drug carriers [114]. Niosomes are a promising vehicle for drug delivery, and since they are non-ionic, they are less toxic and improve the therapeutic index of drugs by restricting their action to target cells. The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, trapped volume, surface charge and concentration can control vesicle characteristics. The vesicles may act as a depot, releasing the drug in a controlled manner.

Niosomes are osmotically active, stable and increase the stability of the entrapped drug. They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration.

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Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in an external non-aqueous phase. Niosomes have been proposed for a number of potential therapeutic applications, i.e., as immunological adjuvants [115], anticancer and anti-infective drug targeting agents [116,117], carriers of anti-inflammatory drugs [118] and as diagnostic imaging agents [114]. In addition, niosomes are versatile carrier systems and can be administered through various routes. Particular efforts have been aimed at using niosomes as effective transdermal drug delivery systems [119,120].

2.5. Cyclodextrin

Cyclodextrins (CDs) are unique molecules with 'pseudo-amphiphilic' structure, and several members of this family are used industrially in pharmaceutical and allied applications. The enzymatic degradation of starch by glucosyltransferase generates cyclic oligomers of α-1,4-D-glucopyranoside, or CDs. CDs with lipophilic inner cavities and hydrophilic outer surfaces are capable of interacting with a large variety of guest molecules to form non-covalent inclusion complexes [121]. CDs have an internal hydrophobic domain that can accommodate poorly water-soluble molecules, while the outer hydrophilic surface facilitates its solubility in the aqueous environment [122,123]. They have been widely exploited for drug delivery and used in the preparation of various delivery vehicles, such as liposomes, microspheres, microcapsules and NPs.

CDs enhance bioavailability of insoluble drugs by increasing drug solubility and dissolution. They also increase the permeability of insoluble, hydrophobic drugs by making the drug available at the surface of the biological barrier (e.g., skin and mucosa) from whence it partitions into the membrane without disrupting the lipid layers of the barrier. In such cases, it is important to use just enough CD to solubilize the drug in the aqueous vehicle since an excess may decrease drug availability [124]. Cyclodextrins can also enhance drug bioavailability by the stabilization of drug molecules at the biomembrane surface. For example, CD-enhanced insulin bioavailability after nasal administration is partly due to this stabilizing effect [125]. Sublingual drug delivery is one of the most efficient ways to bypass hepatic first-pass metabolism [126], whereby the drug enters the systemic circulation by dissolving in the mucosa. In the sublingual formulations, the complexation of poorly water-soluble drugs with cyclodextrin has been shown to increase the bioavailability of various lipophilic drugs [127].

Cyclodextrin has played a very important role in the formulation of poorly water-soluble drugs by improving the apparent drug solubility and dissolution. Cyclodextrins are 'enabling' vehicles and can be used for oral and i.v. delivery. When they are used as vehicles for oral administration, CDs enhance the bioavailability of insoluble drugs by molecular dispersion, protection from degradation, and delivery to the surface of the intestinal wall. When given as parenteral vehicles, they serve as solubilizers for complex hydrophobic drugs without altering their pharmacokinetic properties [122]. Moreover, drugs are associated by non-specific hydrophobic forces and can easily dissociate at sites of greater affinity, i.e., at the lipid-rich surface of the intestinal wall after oral administration [128], or after contact with plasma proteins when administered i.v. [123].

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2.6. Implant delivery system

Implants of drug-loaded polymers, either as millirods, pellets or microspheres, are able to deliver drugs for prolonged periods. The benefits of this subcutaneous implantation include greater assurance of patient compliance, which then leads to better therapeutic outcome, particularly for chronic medication. This approach is well recognized for contraception and hormonal therapy [129–132]. Two types of polymeric delivery systems are being used: nondegradable and biodegradable polymeric matrices.

Non-degradable biomatrices are composed of either silicone or poly(ethylene-co-vinyl acetate) [133]. The Norplant delivery system uses this approach for contraception [134]. Vadhanam et al. have used the system to deliver ellagic acid in a mammary tumorigenesis model and shown effectiveness while delivering 130-fold less compound via silastic implants compared to dietary route (500 ppm), during a 28-week treatment period [135]. Even though this approach has the potential to deliver over prolonged time periods, risks include mechanical failure that may lead to dose dumping, in the case of reservoir systems, and continuous dose drops, in the case of solid-drug distributed matrices. The other issue related to this system is the potential for fibrous growth around the implants, sometimes making it difficult to remove them at the end of the treatment period.

A biodegradable polymeric system overcomes some of the drawbacks of a non-degradable matrix system. There are several polymers used, the most common ones being poly(lactide-co-glycolide) (PLGA), polyanhydrides and PCL. PLGA and PCL undergo bulk erosion resulting in lower molecular weight polymeric chains, while polyanhydride undergoes surface erosion [136]. PLGA degrades more quickly than PCL, and hence it is preferred for shortterm treatments. The implants are in the form of rods, pellets or microspheres. Microspheres have been more commonly used for testing delivery of chemotherapeutic drugs, because they may be injected directly into solid tumors [137,138] and at sites that are not easily accessible, like brain [139]. Hydrogels are another form of biodegradable, polymeric formulation that gel at body temperature after injection [140].

Gupta and co-workers [141] have used PCL in chemopreventive approaches. They have effectively standardized the delivery of multiple chemopreventive agents and demonstrated that release is a combination of passive diffusion and polymer degradation. Since the rate of compound release from the implants depends on its solubility and binding capacity to the implant matrix, long-term release at levels as high as approximately 840 μg per day in the case of resveratrol, and as low as 20 μg per day for luteolin, was achieved [141]. Implantable delivery can provide homogeneous drug distribution and stabilization, as shown for curcumin by differential scanning calorimetry and X-ray diffraction studies [142]. The implants were also shown in animal models to have no associated systemic toxicity [143].

Bansal et al. demonstrated increased bioavailability of curcumin when delivered by polymeric implants. The plasma levels were 9 nM on day 1, decreased to 4 nM on day 4, and reached approximately 543 pM by 3 months by the implant route. By oral delivery, based on 1000 ppm in the diet, only 815 pM was detected on day 4, this decreased to 543 pM by day 12, and levels were undetectable at 3 months (detection limit of 340 pM) [144].

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Enhanced bioavailability has also been shown by the same group for polyphenone E [145] and punicalagin [146].

Although the polymeric implants can be used for sustained short- or long-term release of chemopreventive agents with enhanced bioavailability, these implants cannot be used for heat-sensitive compounds, as preparation of the loaded delivery system requires exposure to high temperatures. Aqil et al. have recently developed an improved method for preparation of multilayer coated implants. Withaferin A delivery by both coated implants and intraperitoneal injections showed efficacy in reducing tumor growth in a lung-cancer xenograft model, but the intraperitoneal route was effective only when twice the dose of withaferin A was used [141,147]. An interesting approach using the implant delivery system was also applied to mimic carcinogen exposure in humans, and this could be used as an ideal model system to test for carcinogenic activity of compounds. Most chemical carcinogenesis studies use high-bolus doses, which are not at all in the realistic range of human exposure. Benzo[*a*]pyrene delivered by an implant system achieved levels of tissue DNA adducts similar to those found in population studies [148].

Desai et al. have also demonstrated use of polymer delivery systems for chemoprevention *in vitro* and *in vivo* by black raspberry extract embedded in PLGA [149], and for release of N-acetyl cysteine [150] and 2-methoxy estradiol [151] *in vitro.*

3. Future approaches

Chemopreventive agents have most often been screened and tested using characterized and controlled cellular environments. The inability to achieve effective concentrations in target tissues *in vivo* has resulted in the failure to demonstrate effectiveness of many compounds that had previously shown promising *in vitro* results. Chemopreventive agents that show promising results in cell culture and other *in vitro* models must first be screened for bioavailability, biodistribution and bioaccumulation before further detailed in-depth *in vitro* studies are carried out. For a successful chemoprevention strategy, bioaccumulation of sufficient levels over relatively long time periods is essential for achieving biological effects. Oral delivery leads to undesired dose spikes and, depending on the half-life of the compound, clearance in a few hours. The complexity of biological challenges to survival of the chemopreventive agent (i.e., surviving low gastric pH, intestinal bacterial metabolism, liver first-pass metabolism, and clearance everywhere along the way) can lead to a low dose at the actual site of action. This challenge can be addressed by slow- or sustained-delivery systems, where the balance between all these biological steps will lead to a constant concentration in the systemic circulation that will facilitate bioaccumulation at the site of action. Study of delivery systems has most frequently been focused on chemotherapeutic uses. If these delivery approaches are used and extended for study of chemopreventive agents, the field of chemoprevention will make progress in leaps and bounds.

Furthermore, the chemopreventive agents should be tagged according to the target organs where they bioaccumulate at the highest levels. For example, if a compound is found to bioaccumulate at highest concentration in prostate and found in relatively lower levels in other organs, then the chemopreventive agent should be tagged as a prostate

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chemopreventive agent. This can be achieved only by exhaustive tissue distribution studies for every compound, which is a daunting task. Such select agents should then be tested *in vivo* for efficacy, toxicity and viability before taking the agent to a clinical trial. Choosing a wrong target organ for a chemopreventive agent, where it does not bioaccumulate significantly, may lead to system toxicity, since increasing the dose in an attempt to achieve efficacy at the wrong target will lead to overly high bioaccumulation at its preferential organ.

It is also important that the chemopreventive agent be a non-nutrient phytochemical. Using essential micronutrient as a chemopreventive agent may tip the balance of other influencing and unidentified micronutrients, which may lead to deleterious effect [10]. However, since cancer is multifactorial, a single agent may not achieve effective chemoprevention. Hence, multiple agents identified to target multiple and overlapping pathways, additively or synergistically, could be delivered using systemic delivery approaches. The benefit of these delivery approaches is that it would achieve desired dose of multiple agents at the target organ. Gupta and co-workers have delivered four different compounds (curcumin, Green Tea Polyphenols, punicalagin and Diindolylmethane) using four implants in a single animal and demonstrated the feasibility of delivering multiple drugs [152].

It is thus clear that if target organs for bioaccumulation of chemopreventive agents are identified and delivery systems are developed to increase stability and half-life, tremendous progress can be achieved in the field of preclinical chemoprevention. This will also provide convincing evidence for clinicians and generate professional interest in chemoprevention clinical trials. However, due to lack of appropriate biomarkers and defined endpoints in primary chemoprevention, future clinical trials should focus on secondary and tertiary chemoprevention, where defined endpoints and biomarkers are more solidly established. In addition, use of established chemopreventive agents that do not interfere with traditional chemotherapies, as adjuvant will provide much-needed progress for the field of chemoprevention.

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