Nutraceutics and Delivery Systems

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Dedicated to the Godfather of the “Juliano Family” I would like to express my deep gratitude to Professor Juliano for adopting me as a member of the “Juliano family” His astute comments continually inspire me and guide the way for a stray sheep. Research requires persistent motivation, and his great achievement of a liposome formulation is a strong incentive to continue this difficult and painstaking task. His constant passion and strenuous efforts spur me and many young scientists. I would like to congratulate him on his enormous scientific achievements in the drug targeting area.

Medical treatment has been shifted to being more prophylactic as a recent trend. Postgenomic research has unveiled the fact that nutritional intervention has been strongly associated with genetic expressions, which are responsible for a variety of biological functions. Based on these findings, the prophylactic effects of dietary supplement and nutrient have been enthusiastically investigated. Preventing or retarding the onset of diseases has become a more attractive and cost effective strategy in the medical arena. Among other approaches to prevent diseases, antioxidants, which are found in many phytochemicals, have received much attention. However, most natural antioxidants such as α-tocopherol, ascorbic acid and others are biologically unstable, poorly soluble in water, and poorly distributed to target sites. Because of these shortcomings further prophylactic applications of dietary supplements have stagnated. This is partially due to a lack of basic awareness of drug delivery system for dietary supplements and nutrients. In this article, we strongly advocate serious consideration of the bioavailability of dietary supplements. Currently, there are some challenging works to improve their bioavailability using delivery systems such as liposomal formulations. We will discuss the target molecules of dietary supplements for prevention of diseases and also introduce the pioneering works of delivery systems for dietary supplements to promote their therapeutic value.

Keywords: Antioxidants; Ascorbic acid; Postgenomic; α-Tocopherol

INTRODUCTION

In addition to laborious work and the expectation of serendipity in the search for pharmacologically active compounds, pharmaceutical companies are now demanding theoretical approaches with high-speed and less cost. Once the target gene sequence responsible for a certain disease is known gene-based medicines such as antisense DNA oligonucleotides and siRNA become a rational method of down regulating specific proteins. Since the decoding of the entire human genome sequences (Lander et al., 2001; Venter et al., 2001), gene-based medicine has leap into the mainstream of the pharmaceutical arena.

Recently, medical treatment has shifted to being less invasive and more prophylactic and alternative sources for new therapeutic molecules are being investigated. Since conventional drugs are often not preventive, complementary and alternative medicine (CAM) have been re-evaluated by medical professionals as potential therapies. Clinical studies of herbal medicine as CAM demonstrate supportive evidence for their empirical efficacy (Pitter and Ernst, 2000; Akase et al., 2003; Xu et al., 2003). Although several CAM clinical trials have been conducted, most of these studies have not fully elucidated CAM mechanisms of action and have rarely provided well-documented evidence of their therapeutic effect (De Smet, 2002). Thus, as a general guideline, clinicians should prescribe only herbal medicine with well established activities (De Smet, 2002).

Systematic investigations and meta-analysis also support the efficacy of some dietary supplements (Reginster et al., 2001; Rapport and Lockwood, 2002). Furthermore, the influence of nutrient and dietary supplements on stages of diseases at a genetic level can be accessed by means of a DNA microarray.
Gene alterations triggered by nutrients and dietary supplements have been actively investigated (Daniel, 2002; Elliot and Ong, 2002; German et al., 2003). Postgenomic approaches to dietary supplements open a new paradigm shift in preventive medicine such as dietary cancer prevention.

Among these, antioxidants, which are found in many phytochemicals, have received the greatest attention. Many diseases are related to an imbalance between oxidants and antioxidants. Unfortunately, most phytochemicals are biologically unstable, poorly soluble in water and poorly distribute to target sites. Because of these shortcomings, further therapeutic applications of dietary supplements have stagnated. Currently, there is little relevant work investigating the bioavailability of dietary supplements. Because of the limited understanding of the bioavailability and delivery systems in nutritional science, dietary supplements might be taken in excessive amounts or delivered to unwanted sites. As a result, insufficient efficacy and interference with the metabolism of conventional drugs can occur.

In this article, we strongly advocate serious consideration of the bioavailability of dietary supplements (Fig. 1) to optimize their use. Currently, there is some novel methods to improve the bioavailability of CAM, for example, the use of liposomal formulations. However, the underlying concept of using delivery systems for CAM is not fully documented. Here we consider important factors relating to delivery systems for CAM, and also review the current delivery methods of CAM to increase their therapeutic value.

TARGET MOLECULES FOR DIETARY SUPPLEMENTS

DNA microarrays can simultaneously investigate a set of thousands of gene alternations, offering a powerful method of addressing the impact of CAM treatment (Lipshutz et al., 1999). For example, Lee, using high-density oligonucleotide arrays, demonstrated caloric restriction in mice retarded the effect of aging on several biochemical processes (Lee et al., 1999). Weindruch also reported microarray profiling of gene expression in mice with caloric restriction (Weindruch et al., 2001). According to their reports, the largest differential expression between young and aging animals was in the enzyme skeletal muscle mitochondrial sarcomeric creatine kinase. Mitochondrial sarcomeric creatine kinase is a critical enzyme for peroxynitrite-induced inactivation (Stachowiak et al., 1998). The production of reactive oxygen species (ROS) in mitochondria increases with aging (Sohal and Windruch, 1996). As a result, mitochondrial sarcomeric creatine kinase may increase to compensate for the response of upregulated ROS production

Caloric restriction also restores protein metabolism, energy metabolism and mitochondrial function (Weindruch et al., 2001). Caloric restriction retards the aging process by altering gene expressions related to protein metabolism and energy metabolism. Weindruch et al. investigated 6347 genes representing 5–20% of the mouse genome (Weindruch et al., 2001). A more complete analysis of gene expression patterns could unveil the complicated biophysiology of aging.

FIGURE 1 Concept and targeting site for dietary supplements.
Target molecules for dietary supplements have been investigated, suggesting this might be a more rational approach for nutritional intervention in disease prevention.

REGULATION OF OXIDATIVE STRESS AND NATURAL ANTIOXIDANTS

Oxidative stress is associated with many conditions such as Alzheimer’s disease, diabetes, coronary heart disease, atherosclerosis, cataracts, cancer, viral infection, inflammation, aging and cerebral ischemia (Han and Meydani, 2000; Butterfield et al., 2003; Griending and FitzGerald, 2003; Vergely et al., 2003). The level of amine oxidases is also related to tumor malignancy. The balance between oxidant and antioxidant enzymes plays many important roles in carcinogenesis (Pietrangelo and Mondovi, 2004). To attain the therapeutic value of a natural antioxidant such as α-tocopherol an appropriate drug delivery system is required. The following experiments demonstrate a successful antioxidant strategy.

Oxidative damage plays an important role in ischemic brain damage. A therapeutic reduction of oxidative brain damage would be beneficial to recovery from cerebral ischemia. Restoration of blood flow brings oxygen and nutrients into the damaged brain, however, acute restoration of blood flow also damages brain tissue by the generation of ROS (Birnbaum et al., 1997). Tissue plasminogen activator (TPA) is the only therapeutic agent for acute ischemic stroke approved by the Food and Drug Administration. However, TPA has limitations and risks because of the increased risk of intracerebral hemorrhage and mortality. Pahlmark et al. demonstrated that an antioxidant strategy is useful in diminishing ischemic tissue damage (Pahlmark and Siesjö, 1996). Although ascorbic acid is a classic antioxidant, it penetrates the blood–brain barrier (BBB) poorly. Huang et al. used the oxidized ascorbic acid form, dehydroascorbic acid (DHA), to investigate its anti-ischemic effect (Huang et al., 2001). DHA, given intravenously in murine cerebral ischemia, was readily transported across the ischemic BBB and successfully reduced the infarct volume in the ischemic damaged brain. This study demonstrated that DHA was an ideal pro-drug to achieve therapeutic efficacy of ascorbic acid.

Hyperglycemia generates excess ROS in the tissue, triggering oxidative stress, as shown in Fig. 2. Dincer et al. suggested that antioxidative enzymes such as superoxide dismutase (SOD) were augmented in the liver (Dincer et al., 2002). Previous experimental data suggested that lipid peroxidation occurred in the liver of diabetic animal models. Earlier experiments showed that liver SOD activity is also positively correlated with blood glucose level in diabetes models. This suggested that oxidative stress stimulated ROS-scavenging enzymes to compensate for the imbalance between oxidants and antioxidants.

Natural antioxidants such as α-tocopherol and tea catechin are beneficial in attaining an antioxidative effect in vivo.

Tea contains polyphenols, which have a variety of biological functions (Park and Dong, 2003). Among tea polyphenols, epigallocatechin-3-gallate in green tea and theaflavin-3',3'-digallate in black tea are effective anti-cancer factors. Also some tea polyphenols such as epigallocatechin, theaflavin and thearubigin have direct and indirect antioxidative actions. They may act indirectly as antioxidants through: (1) inhibition of Activator protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B), (2) inhibition of prooxidant enzymes and (3) induction of antioxidant enzymes (Frei and Higdon, 2003). AP-1 and NF-kappa B are transcriptional factors playing a critical role in tumorgenecity, and are also strongly related to oxidative stress. It is suggested that tea polyphenols prevent tumorgenecity by targeting AP-1 and NF-kappa B (Park and Dong, 2003). NF-kappa B is activated by oxidative stress under the conditions of hyperglycemia. Many antioxidants such as pycnogenol, tea catechin and resveratrol block the activation of NF-kappa B (Manna et al., 2000; Peng et al., 2000; Shi et al., 2000). Suppression of activated NF-kappa B could be a promising target of an antioxidant strategy in diabetic mellitus. To assess the actions of α-tocopherol on cytokine levels in α-tocopherol-supplemented individuals, a human cytokine array was used. α-Tocopherol affected several cytokine levels; especially chemotactrant protein-1 (MCP-1) was markedly reduced. This result suggests that MCP-1 might be an important target of oxidative stress.
α-tocopherol (Lin et al. 2002). Using human intestinal epithelial Caco-2 cells Shimizu et al. (2000), demonstrated that tea catechins regulate intestinal glucose transport through specific transporters, SGLT1, SGLT2 and SGLT5. Glucose transporters could also be target molecules for tea polyphenols.

**IMPROVEMENT OF SOLUBILITY AND CELLULAR TRANSPORT**

As shown in Fig. 3, there are several steps for improving the bioavailability of dietary supplements including: (1) enhancement of biological stability including solubility, (2) improvement of cellular transport and (3) release of the active component inside the cell. Since several dietary supplements have poor bioavailability, they can be distributed to unwanted sites or eliminated rapidly from the body. Several chemical approaches have been used to address the limited water solubility of phytochemicals such as α-tocopherol. For example, astaxanthin is an oxygenated carotenoid that demonstrates improved aqueous solubility.

Besides water solubility, chemical stability can also be a challenge. For example, ascorbic acid is unstable under heat and oxidation, so stable derivatives in vitro are needed to maintain the in vivo biological activities. Tai et al. have synthesized a series of ascorbic acid derivatives and measured their pharmacokinetics after oral and intravenous administration (Tai et al., 2002). After oral administration, these derivatives were hydrolyzed into ascorbic acid through the process of absorption and were able to maintain their antioxidative activities.

**TABLE I** Delivery systems applied for dietary supplements

<table>
<thead>
<tr>
<th>Dietary supplements</th>
<th>Delivery methods</th>
<th>References</th>
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<tbody>
<tr>
<td>Soy isoﬂavone</td>
<td>Low density lipoprotein</td>
<td>Meng et al. (1999)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Pro-drug</td>
<td>Taka et al. (2002)</td>
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<tr>
<td>Vitamin K</td>
<td>Pro-drug</td>
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<tr>
<td>Hydroquinone</td>
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<td>Omega-3 unsaturated fatty acid ethyl esters</td>
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<td>Melatonin</td>
<td>Chitosan microspheres</td>
<td>Dini et al. (2003)</td>
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<tr>
<td>Astaxanthin</td>
<td>Vehicles for dermal delivery</td>
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<tr>
<td>CoQ10</td>
<td>Sustained-release formulation</td>
<td>El-Gibaly et al. (2003)</td>
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<tr>
<td>Vitamin E</td>
<td>Chitosan microcapsules</td>
<td>Ficarra et al. (2002)</td>
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<tr>
<td>Quercetin</td>
<td>Cyclodextrins</td>
<td>Hirasawa et al. (2002)</td>
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<tr>
<td>Rutin quercetin</td>
<td>Hydroxypropylcellulose</td>
<td>Mandal et al. (2002)</td>
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<tr>
<td>Astaxanthin</td>
<td>Mannosylated liposome</td>
<td>Lauro et al. (2002)</td>
</tr>
<tr>
<td>CoQ10</td>
<td>Chitosan microspheres</td>
<td></td>
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<tr>
<td>Vitamin E</td>
<td>CMC-XL, carboxymethylstarch, polyvinylpyrrolidone, HPMC</td>
<td></td>
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<tr>
<td>Sodium ascorbic phosphate</td>
<td>Lipid based formulation</td>
<td>Mercke et al. (2003)</td>
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<td>Vitamin E</td>
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<td>Quercetin</td>
<td>Topical microemulsion</td>
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<tr>
<td>Rutin quercetin</td>
<td>Marine lipid-based liposomes</td>
<td>Nacka et al. (2001)</td>
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CMC-XL, cross-linked sodium carboxy methylcellulose; HPMC, hydroxypropylmethylcellulose.

Current delivery systems for dietary supplements are summarized in Table I. Phytochemicals have a variety of biological functions such as chemoprevention, antimicrobial activity, prevention of apoptosis and anti-inflammatory activity. However, the lack of solubility of phytochemicals has limited their use as therapeutic agents.

**FIGURE 3** Conceptual DDS for dietary supplement. There are three major steps in the drug delivery system of dietary supplement: (1) enhancement of biological stability including solubility, (2) improvement of cellular transport and (3) release of the active component inside the cell.
α-Tocopherol is thought to be one of the strongest antioxidants and has received a great deal of attention in terms of therapeutic efficacy. It has been utilized for preventing and reducing oxidative stress. However, it is highly viscous, barely soluble in water and is readily oxidized in vitro. To facilitate the potential antioxidative activity of α-tocopherol, a proper delivery system is needed. Generally, acetate or succinate esters of the α-tocopherol have been utilized for clinical use. Takata applied a pro-drug formulation for α-tocopherol to overcome these shortcomings (Takata et al., 2002). His group determined that the hydrochloride salt of D-α-tocopherol N,N-dimethylaminoacetate (TDMA) was the most promising pro-drug for parenteral delivery. TDMA was successfully delivered to the liver and revealed efficacy after i.v. administration. Furthermore, TDMA also showed lower toxicity (Takata et al., 2002).

Another drug delivery system for α-tocopherol under investigation is a thermo-sensitive sol-gel system, which releases the drug when the temperature rises. A thermo-sensitive copolymer consisting of poly (2-ethoxyethyl vinyl ether) and poly (hydroxyethyl risd. A thermo-sensitive copolymer consisting of poly (2-ethoxyethyl vinyl ether) and poly (hydroxyethyl vinyl ether) (EOVE200-HOVE400) was synthesized and applied to α-tocopherol (Ishida et al., 2003). α-Tocopherol is released from EOVE200-HOVE400 when the temperature changes from 10 to 30°C, thus improving the solubility.

This is an example of successful improvement of cellular transport using the prodrug concept. The concept of delivery efficiency is shown in Fig. 3. Captisol is a cyclodextrin formulation that increases the aqueous solubility of astaxanthin. However, the amount remains insufficient for the clinical application; in addition captisol itself has adverse reactions. The development of appropriate delivery carriers still remains a goal.

LIPOSOMAL FORMULATION

Genistein and daidzein, which are compounds extracted from soybean, demonstrate female hormonal activities. These active components exert beneficial health effects on menopausal symptoms. Because of their high lipophilicity and low water solubility, their usage is limited. One way to increase drug transport is to use an endogenous carrier system. Low density lipoprotein (LDL) is efficiently taken up via the LDL receptor in most cells. Soy flavonoids are successfully incorporated into LDL particles (Meng et al., 1999). Daizein 4',7-dilinoleate was incorporated into LDL particles, and showed a strong antiproliferative effect on U937 cells. Since 4'-oleate of genistein and daidzein contained in LDL did not reduce the proliferation of U937; the release rate of compounds from LDL may affect overall antiproliferative effects.

Minko et al. prepared a liposomal formulation of α-tocopherol to evaluate its efficacy in a lung model of hypoxia. Liposomal α-tocopherol was intratracheally administered 30 min after exposure to hypoxia in rats. α-Tocopherol significantly inhibited lipid peroxidation in lung tissues and suppressed lung damage. As a result, breathing patterns, oxygen diffusion and lung gas exchange were improved (Minko et al., 2002). α-Tocopherol incorporated into liposomes successfully prevents hypoxic lung damage through its antioxidant and its anti-apoptotic effects.

ALTERNATIVE DELIVERY ROUTES

In addition to oral or parenteral administration of dietary supplements, alternative administration routes are being investigated, for example, the transdermal use of melatonin. Since melatonin has a short biological half-life and is subjected to the first pass effect, an administration route other than oral is needed. Dermal application of melatonin is an attractive route for local effects. Kitwai et al. (2002) used several vehicles including isopropyl myristate, lauroglycol FCC and ethanol to study the diffusion of melatonin through porcine skin. The flux of melatonin with myristate, lauroglycol FCC, and ethanol was significantly higher than that with water, whereas it was significantly lower with Labrasol, propylene glycol and mineral oil. These results suggest that vehicles with a higher solubility of melatonin show a low permeability coefficient. Other studies have demonstrated that sustained-release melatonin significantly improves the quality of sleep and vitality in patients with sub-syndromal seasonal affective disorder in a randomized controlled trial; this suggests that long acting activity of hormones functioning like melatonin requires sustained release formulation. Also, various saturated fatty acids have been investigated to enhance the skin permeation of melatonin (Leppamaki et al., 2003). Saturated lauric acid (C12) and unsaturated oleic acid (C18) markedly enhanced the skin permeability of melatonin (Oh et al., 2001).

Besides the use of chemical penetration enhancers, direct physical methods, such as iontophores, can also be utilized. As mentioned previously, ascorbic acid is biologically unstable and skin is impermeable to it. Therefore percutaneous administration of ascorbic acid is very limited. In order to improve the percutaneous penetration of ascorbic acid, Ebihara et al. applied an iontophoresis technique (2003). After topical administration of [14C]-labeled ascorbic acid to rats, epidermal, dermal and blood compartment radioactivities were compared in the presence/absence of iontophoresis. Iontophoresis was shown to enhance the percutaneous penetration of [14C]-labeled ascorbic acid (Ebihara et al., 2003).

Antioxidants are expected to protect the skin from oxidative damage brought about by sun exposure. Sunscreens containing antioxidants such as α-tocopherol are available. To enhance the permeability of α-tocopherol, many delivery systems have been compared in vitro using micro-Yucatan pig skin model (Rangarajan...
and Zatz, 2003). Among other systems studied, a microemulsion containing isopropyl myristate showed a potent delivery effect, although future studies are required (Rangarajan and Zatz, 2003).

CONCLUSION

We have described the current delivery systems for dietary supplements. There are several approaches available, such as: (1) utilization of pro-drugs, (2) improvement of solubility and (3) enhancement of cellular permeability. Since most natural antioxidants have limited water solubility, improvement of the solubility in vitro enhanced the overall efficacy. Takata et al. investigated several prodrugs of α-tocopherol and showed their potency for parenteral use (Takata et al., 2002). The pro-drug form of ascorbic acid was shown to enhance its permeability through the BBB (Huang et al., 2001). Thus, appropriate delivery methods for dietary supplements open a new possibility by enhancing their therapeutic value. For instance, antioxidants block the activation of NF-kappa B, which may eventually suppress conditions associated with diabetes (Fig. 2).

Another incentive to develop delivery methods for dietary supplements would be attributable to recent medical trends, which have shifted to disease prevention. Among various approaches, nutritional genomics is the cornerstone of disease prevention. The effort to improve the quality of life embraces research on nutraceutics and functional foods. According to the new definition of nutrients, nutrients are needed to maintain cell differentiation, renewal, repair, defense, recruitment of signaling molecules, catalytic reactions and promotion of assembly of mechanistic structures (Young, 2001). Some dietary supplements are considered to be the primary source for prevention or reduction of the risk factors for several diseases (Lopez-Varela et al., 2002). However, the data regarding their bioavailability is not well established.

Without prejudice, continuous challenge to investigate the clinical efficacy and the mechanism of their active components is needed. Early pioneering work has successfully enhanced the bioavailability of dietary supplements and it has been demonstrated that delivery systems can increase their therapeutic value; however, new, more practical delivery systems are needed. Through this approach, dietary supplements would be more actively used for the prevention of diseases.

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References


