

Delivery of the vitamin E compound tocotrienol to cancer cells

Tocotrienol, a member of the vitamin E family of compounds, is currently receiving increased attention owing to its highly promising anticancer effects. However, its potential in cancer therapy is limited by its poor bioavailability and its inability to specifically reach tumors at therapeutic concentrations after intravenous administration. In order to address these problems, various delivery strategies have been proposed, such as the inclusion of tocotrienol in γ -cyclodextrins, prodrugs and emulsions, and entrapment in lipid nanoparticles and vesicles. Among these approaches, we have demonstrated that the entrapment of tocotrienol within vesicles bearing transferrin, whose receptors are overexpressed on numerous cancer cells, significantly improved the uptake by cancer cells overexpressing transferrin receptors. Consequently, the intravenous administration of tocotrienol entrapped in transferrin-bearing vesicles led to tumor regression and even complete tumor suppression in some cases in a murine tumor model, as well as improvement of animal survival. Transferrin-bearing vesicles are therefore highly promising for the delivery of tocotrienol to cancer cells *in vitro* and *in vivo*, and should be further investigated to optimize the anticancer therapeutic effect of tocotrienol.

Vitamin E refers to a family of compounds that is divided into two subgroups, namely tocopherol and tocotrienol. Tocopherol is most commonly found in vegetable oils, animal fats and nuts, whereas tocotrienol is primarily derived from only a few vegetable fats, such as palm oil, rice bran, oat, wheat germ and barley [1,2].

Structurally, both tocopherol and tocotrienol consist of a chromane ring linked to a 16-carbon phytyl side chain (**FIGURE 1**) [3]. However, the difference between tocopherol and tocotrienol lies in the saturation of the phytyl chain and the degree of methylation of the chromane ring. The phytyl side chain of tocopherols is fully saturated, unlike that of tocotrienol, which contains three *trans* double bonds. Furthermore, the four isomers of tocopherol and tocotrienol differ in the number and position of methyl groups attached to the chromane head.

Although tocopherol and tocotrienol share similar chemical structures, only tocotrienol displays promising anticancer activity. Its tumor suppressive effects have been demonstrated in many cancer cell lines *in vitro*, including breast, colorectal, pancreatic, gastric, liver, lung and prostate cancer [4].

In vivo, tocotrienol solution has been used mainly for oral administration or as a therapeutic adjuvant.

Oral intake of tocotrienol led to inhibition of tumorigenesis in B16 murine melanoma models [5]. However, this administration strategy is limited for therapeutic purposes by the fact that oral absorption of tocotrienol into the circulation is mostly

mediated by carrier-transporter systems that display saturation and down-regulation as a result of exposition to high concentrations of tocotrienol [6].

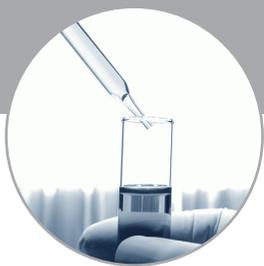
The use of tocotrienol in combination therapy with chemotherapeutic drugs such as celecoxib, tamoxifen and statins resulted in synergistic antiproliferative effects [7–9]. This therapeutic response was more pronounced when tocotrienol was administered with drugs that have complementary anticancer mechanisms of action.

Tocotrienol has been reported to exert its anticancer effects through various mechanisms, such as induction of apoptosis, activation of p53, decrease of oxidative stress and modulation of the *Bax/Bcl-2* ratio. It is also able to down-regulate the expression of the VEGF receptor and to block intracellular VEGF signaling, leading to inhibition of angiogenesis [3,10,11]. Tocotrienol also inhibits numerous enzymes related to cancer cell proliferation, such as DNA polymerase and telomerase and the NF- κ B activation pathway, thus resulting in the potentiation of apoptosis [3,4,10,11]. This multiplicity of anticancer effects therefore makes tocotrienol a very promising therapeutic molecule.

However, the potential of tocotrienol in cancer therapy is limited by its inability to specifically reach tumors at therapeutic concentrations after intravenous administration. Given the antiproliferative properties of tocotrienol, it is crucial to deliver this therapeutic drug specifically to its site of action. In addition, therapeutic formulation of tocotrienol is

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complicated by its unfavorable physicochemical properties (highly viscous oil, nearly insoluble in water and readily oxidized by atmospheric oxygen). When administered as a solution, tocotrienol has poor bioavailability, regardless of the route of administration (parenteral, oral or topical) [12].

In order to address these problems, various delivery strategies have been proposed: inclusion of tocotrienol in cyclodextrins, prodrugs and emulsions, entrapment in lipid nanoparticles, and tumor-targeted vesicles.

Nontargeted delivery strategies

So far, relatively few strategies have been attempted to improve the bioavailability and delivery of tocotrienol. They consist of including tocotrienol in cyclodextrins, prodrugs and emulsions.

Cyclodextrins are cyclic oligosaccharides containing six to eight α -D-glucopyranoside units attached by α -(1,4) glucosidic bonds. Their lipophilic inner cavities and hydrophilic outer surfaces can interact with numerous guest molecules to form noncovalent inclusion complexes. Cyclodextrins have therefore been particularly useful in oral drug delivery for improving the bioavailability of lipophilic drugs by increasing the drug solubility and stability at the absorption site (the GI tract) and in formulation [13]. Previous studies have demonstrated

the improved bioavailability of ketoprofen, griseofulvin and terfenadine drugs by inclusion in cyclodextrins. A cyclodextrin formulation of itraconazole has been commercialized in the USA and Europe [13].

In their studies of inclusion of tocotrienol in cyclodextrins, Ikeda *et al.* [14], as well as Miyoshi *et al.* [15], have chosen to use γ -cyclodextrin, as it shows the highest water solubility among the three existing natural cyclodextrins. A tocotrienol-rich fraction (TRF) of rice bran containing 0.5% α -tocopherol, 0.4% β -tocopherol, 2.8% γ -tocopherol, 0.5% δ -tocopherol, 1.8% α -tocotrienol, 0.4% β -tocotrienol, 65.0% γ -tocotrienol and 4.6% δ -tocotrienol was used in these two studies.

Wistar rats and C57Bl6 mice were subjected to a vitamin E free diet for 28 days, before being orally administered with either TRF as a solution or enclosed in cyclodextrins. Pharmacokinetic studies showed an increase of plasma concentration after the oral administration of TRF included in cyclodextrins. The C_{max} and area under curve values in mice receiving the cyclodextrin formulation were approximately 1.4-fold higher than those after treatment with TRF solution. In addition, Miyoshi *et al.* demonstrated that the mice injected with the cyclodextrin formulation of tocotrienol were less sensible to endotoxic shock induced by injection with lethal

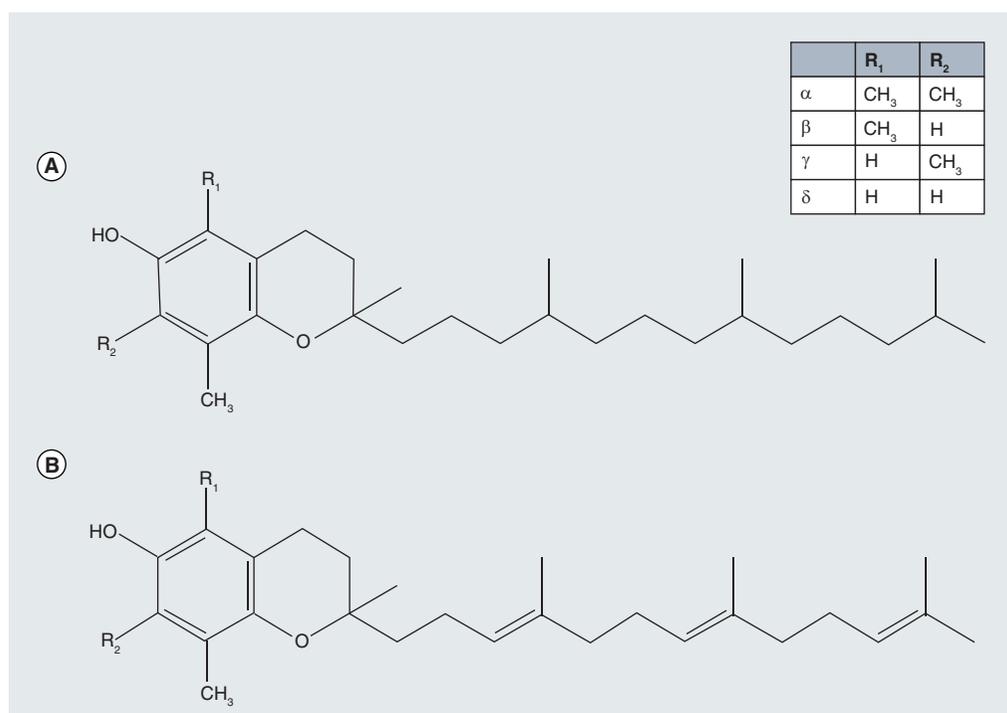
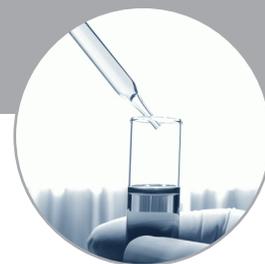


Figure 1. (A) Tocopherols and (B) tocotrienols.



amounts of *Escherichia coli* lipopolysaccharide, compared with the mice having received TRF solution [15]. These results suggest that the inclusion of tocotrienol in cyclodextrins enhance the bioavailability and consequently the bioactivity of tocotrienol.

Another strategy to improve the bioavailability of tocotrienol was to produce hydrophilic prodrugs of tocotrienol. Water-soluble prodrug esters have been widely used for enhancing the aqueous solubility of poorly soluble drugs that contain a hydroxyl group. Ideally, a prodrug is expected to improve aqueous solubility and should be rapidly converted to the active drug *in vivo*. It has previously been demonstrated that esters of γ -tocopherol, which has a phenolic functional group such as tocotrienol, have good water solubility and were able to enhance plasma and liver availability of tocotrienol after intravenous administration in rats, suggesting that similar improvements could be observed with tocotrienol as well.

Akaho *et al.* produced an ester derivative of γ -tocotrienol, namely 2R- γ -tocotrienyl *N,N*-dimethylaminoacetate hydrochloride [16]. This ester showed high solubility and stability in water and was readily converted into the active drug γ -tocotrienol by esterases in liver. The intravenous administration of this prodrug to Sprague–Dawley rats resulted in a rapid increase in the liver, heart, plasma and kidney levels of γ -tocotrienol, thus making prodrugs of tocotrienol interesting formulations to enhance the drug bioavailability.

The poor bioavailability of tocotrienol makes it particularly suitable for self-emulsifying systems to enhance its oral bioavailability. The stability of drugs in the oil phase is increased compared with that of the same drug in aqueous solutions. In addition, emulsions also allow a sustained release of active drugs, resulting in increased efficacy of the treatment. It has been previously demonstrated that the droplet sizes of the emulsion should be as small as possible to increase drug bioavailability.

Tocomin[®] 50% dissolved in soybean oil has been formulated by Yap and Yuen as self-emulsifying systems with the addition of the surfactants Tween 80 and labrasol [17]. Tocomin[®] 50% consisted of 21.6% γ -tocotrienol, 6.4% δ -tocotrienol, 10.7% α -tocotrienol and 10.9% α -tocopherol. These emulsions improved the bioavailability of tocotrienol by approximately two- to three-fold compared with that of a nonself-emulsifying formulation in human volunteers.

Targeted delivery strategies

Three strategies for the tumor-targeted delivery of tocotrienol after intravenous administration have been tested so far.

Passive tumor targeting of tocotrienol and simvastatin co-encapsulated in lipid nanoparticles has been tested by Ali *et al.* [18]. Lipid nanoparticles are particulate systems that possess a solid lipid core matrix able to solubilize lipophilic drugs. These nanoparticles are able to accumulate in tumors, owing to the enhanced permeability and retention effect observed for all particulate systems.

The tocotrienol used for this study was a tocotrienol-rich fraction of palm oil, consisting of 9.8% α -tocopherol, 27.9% α -tocotrienol, 41.4% γ -tocotrienol and 19.2% δ -tocotrienol.

Simvastatin and TRF were both entrapped within the oily compartments of the delivery system. *In vitro*, the nanoparticles of TRF and simvastatin were demonstrated to have an improved antiproliferative effect against +SA breast cancer cell line, compared with reference α -tocopherol nanoparticles (with IC₅₀ respectively of 0.52 μ M and 17.7 μ M) [18].

Prior to this study, we hypothesized that active tumor targeting of tocotrienol by entrapment within transferrin-bearing vesicles could result in a selective delivery of tocotrienol to tumors after intravenous administration, thus resulting in an improved therapeutic efficacy. Transferrin has been chosen as an active targeting moiety, since transferrin receptors are overexpressed in numerous cancers. It was hoped that the combination of active targeting, based on the use of ligands, and passive targeting, based on the accumulation of particulate delivery systems owing to enhanced permeability and retention, would provide a tumor-selective targeting strategy for tocotrienol delivery. Transferrin has previously been used successfully as a tumor-targeting ligand for several drug-delivery systems.

The entrapment of TRF in transferrin-bearing vesicles significantly improved TRF uptake by at least threefold in A431 epidermoid carcinoma, A2780 ovarian carcinoma and T98G glioma compared with TRF solution [19]. Consequently, it led to more than a 100-fold improvement in cytotoxicity in the three tested cell lines compared with TRF solution. *In vivo*, the intravenous administration of TRF entrapped in transferrin-bearing vesicles led to tumor regression and improvement of animal survival in nude mice bearing subcutaneous A431 tumors, in contrast to that observed with



controls [19]. At the end of the experiment, the extended survival of the mice treated with targeted vesicles, control vesicles and free drug was 19, 12 and 2 days, respectively, compared with untreated mice. The most striking effects of TRF entrapped in targeted vesicles were the ability of the drug to induce tumor regression within only 24 h and the powerful tumor regression observed within 10 days. These had good tolerability of the treatment by the mice. This work corresponded to the first preparation of a tumor-targeted delivery system able to encapsulate tocotrienol.

In our follow-up work with transferrin-bearing vesicles, we wanted to enhance and prolong the therapeutic effect of tocotrienol by entrapping this lipophilic drug in multilamellar, rather than unilamellar, vesicles, with the aim of improving drug loading within the lipidic membranes and therefore enhancing the therapeutic efficacy of this system. As previously observed with our Solulan-based delivery system, transferrin-bearing, tocopheryl-based multilamellar vesicles entrapping tocotrienol were able to improve the drug uptake by cancer cells overexpressing transferrin receptors, namely A431, T98G and B16-F10 melanoma [20]. This resulted in an enhanced antiproliferative efficacy *in vitro*, reaching a maximum of 72-fold improvement for A431 cells, compared with the free drug.

In vivo results were improved compared with those observed when using our previous delivery system. The intravenous administration of this novel tocotrienol formulation led to complete tumor eradication for 40% of B16-F10 tumors and 20% of A431 tumors [20]. The tumor regression appeared to follow a different pattern to that previously observed: the inter-individual variability in the response to treatment was more pronounced in this study, but the overall therapeutic effect was more pronounced. This might be due to the involvement of different mechanisms of action of tocotrienol and is currently being further investigated.

Animal survival was improved by more than 20 days compared with controls, for the two tumor models tested. These therapeutic effects potentially make transferrin-bearing vesicles entrapping tocotrienol a highly promising therapeutic system as part of an anticancer therapeutic strategy [20].

Conclusion

The major obstacles for the development of tocotrienol as a successful anticancer drug are the poor bioavailability of the drug as well as its inability to specifically reach tumors at therapeutic concentrations after intravenous administration. Several methods for overcoming these problems are currently under investigation and have already led to significant improvement in tocotrienol bioavailability following oral administration.

The entrapment of tocotrienol within tumor-targeted delivery systems demonstrated its effectiveness *in vivo*. We have recently demonstrated for the first time that a novel tocotrienol formulation can lead to complete tumor suppression after intravenous administration. Tocotrienol entrapped in transferrin-bearing vesicles led to complete tumor suppression for 40% of B16-F10 tumors and 20% of A431 tumors, with long-term survival of the animals. By contrast, 100% of the tumors treated with tocotrienol solution or left untreated were growing for both the tested tumors.

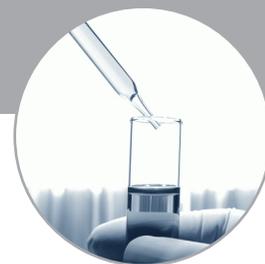
These findings suggest that tumor-targeted tocotrienol could ultimately become a promising therapeutic tool in the treatment of cancer.

Future perspective

While a large amount of research into tocotrienol has been focused on the elucidation of the numerous and extremely diverse mechanisms of action implicated in the anticancer effects of tocotrienol, little has been done regarding the tumor-specific delivery of this drug *in vivo*. It is hoped that the promising results obtained when entrapping tocotrienol within these tumor-targeted delivery

Executive summary

- The vitamin E compound tocotrienol has promising anticancer properties *in vitro*.
- Its use is limited by its poor bioavailability and its inability to specifically reach tumors at therapeutic concentrations after intravenous administration.
- Inclusion of tocotrienol in cyclodextrins, prodrugs and emulsions improved the bioavailability of the drug.
- *In vitro*, the entrapment in lipid nanoparticles improved the antiproliferative efficacy of the drug.
- *In vivo*, the intravenous administration of tocotrienol entrapped in transferrin-bearing vesicles led to tumor regression and even complete tumor suppression in some cases in a murine tumor model, as well as improvement of animal survival.
- Transferrin-bearing vesicles are therefore highly promising for the delivery of tocotrienol to cancer cells *in vitro* and *in vivo*, and should be further investigated to optimize the anticancer therapeutic effect of tocotrienol.



systems will lead to the development of this field of research, in order to reveal the full anticancer potential of this drug. To date, no exact mechanism has been identified as to how tocotrienol works. It will therefore be extremely important to precisely understand how tocotrienol functions, in order to be able to enhance and maintain its therapeutic action against cancer. Such understanding will be paramount to ultimately test this novel agent in preclinical and clinical settings.

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