Development of a novel tocotrienol analogue, tocoflexol, as a radiomitigator

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Abstract

There is a need to develop safe and effective nuclear medical countermeasures that can be used clinically in emergency situations. A major nuclear reactor accident or a nuclear attack could result in catastrophic health consequences to millions of people, a danger which is growing by the day. Currently there are no safe and effective radioprotectors and radiomitigators that can offer multi-organ protection, when administered before or after radiation exposure, respectively. Vitamin E tocotrienols have remarkable radioprotective activity when pre-administered subcutaneously, protecting the hematopoietic, gastrointestinal, and other systems against radiation, with very low toxicity. However, tocotrienols have poor pharmacokinetic properties (low oral bioavailability, delayed subcutaneous absorption, and rapid elimination) that render them unsuitable for radiomitigation when administered after radiation. To overcome the limitations of the tocotrienols, we have used computational techniques to develop a synthetic tocotrienol analogue named tocoflexol. Tocoflexol is designed to have an improved pharmacokinetic profile, suitable for radiomitigation while retaining the powerful therapeutic properties of tocotrienols. Our computational analysis has shown that tocoflexol has superior binding capacity to ATTP, the key transporter that reduces the elimination rate of tocols. Tocoflexol has strong antioxidant properties comparable to tocotrienols, which are key for protecting against radiation, along with rapid cell uptake. Our pharmacokinetic calculations also predict considerably improved oral bioavailability of tocoflexol *in vivo*. Most importantly, we have recently shown that subcutaneous administration of tocoflexol (300 mg/kg) in mice 24 hours prior to lethal total-body irradiation (9.5 Gy) is powerfully radioprotective, providing 100% survival. Tocoflexol is a promising option to develop into an economical and efficacious radioprotectant product.

The University of Arkansas has received patent protection for parts of this research. A potential royalty stream may occur consistent with the University of Arkansas policy.

Hypothesis

Because tocoflexol is designed to have a favorable pharmacokinetic profile compared to conventional tocotrienols (rapid onset and slow elimination), we propose that tocoflexol will be effective as a radioprotectant and radiomitigator.

Methods

- Tocoflexol was designed using computational analysis and molecular modeling to create the optimal tocotrienol analogue that binds to ATTP with the minimal amount of modifications (SYBYL 8.1, PRODRG, and COOT software were used)
- Enantiomerically pure tocoflexol was synthesized from δ-tocotrienol
- Pharmacokinetic modeling estimated the oral bioavailability of tocoflexol compared to other tocols
- Cell uptake assay was used to measure cellular levels of tocols throughout a 24h incubation
- TBARS assay measured the antioxidant potency of tocols in rat liver microsomes
- A mouse model of lethal total body irradiation evaluated the radioprotective efficacy of tocoflexol. Mice were subcutaneously administered tocoflexol 24h before 9.5 Gy radiation and monitored for 30 days

Results

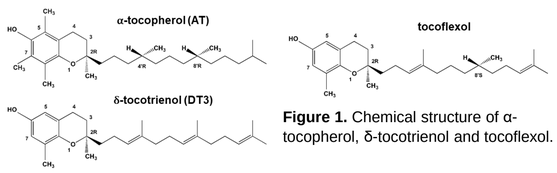

Figure 1. Chemical structure of α-tocopherol, δ-tocotrienol and tocoflexol.

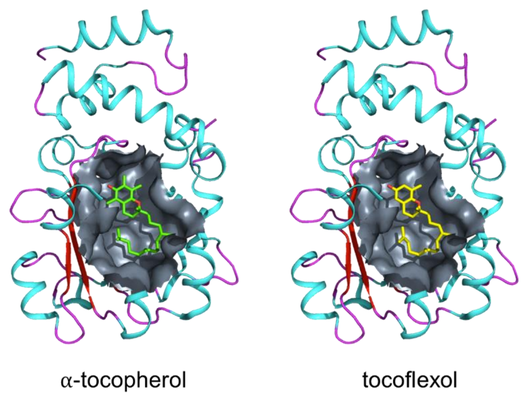

Figure 2. Computational modeling shows binding of α-tocopherol and tocoflexol to the binding pocket of ATTP. Coordinates of ATTP were obtained from PDB entry 1R5L. Topology files for tocoflexol were generated using the program PRODRG. Tocoflexol was fitted in the cavity of ATTP using the program COOT.

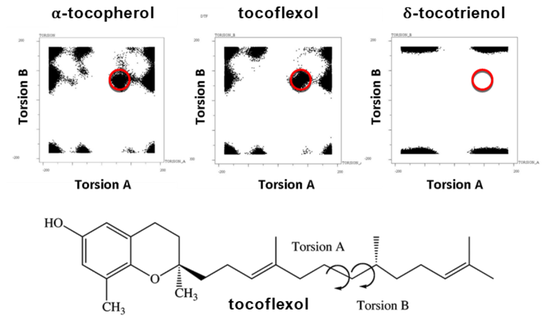

Figure 3. Comparative molecular dynamics simulations analysis. The red circles depict the areas with conformations suitable for binding. Only tocoflexol and α-tocopherol can adapt conformations suitable for high affinity binding with α-TTP, whereas GT3 does not show any conformations in the favorable region.

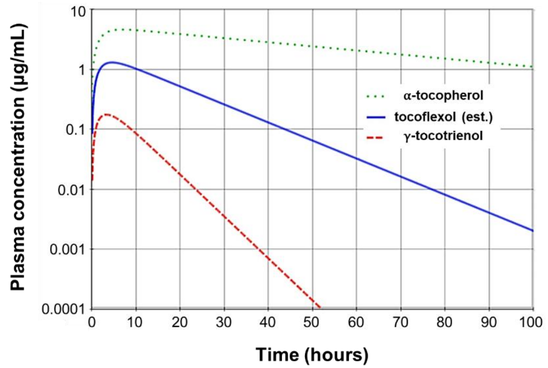

Figure 4. Pharmacokinetic modeling shows estimated time course for plasma levels of tocoflexol after an oral dose. The tocoflexol curve is based on a half-life estimated to be 38% of that of α-tocopherol and an oral bioavailability *F* estimated to be midway between α-tocopherol and γ-tocotrienol.

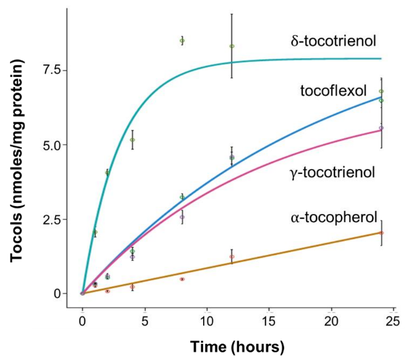

Figure 5. Cell uptake assay shows cellular tocol levels after incubating NSC-34 cells with 5 μM tocols.

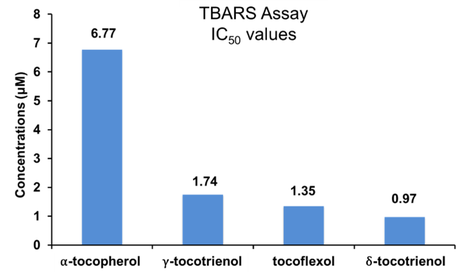

Figure 6. TBARS assay shows IC50 values for inhibiting TBHP-induced lipid peroxidation in rat liver microsomes. Isolated microsomes were incubated with 5 μM tocols in the presence of the TBHP oxidant.

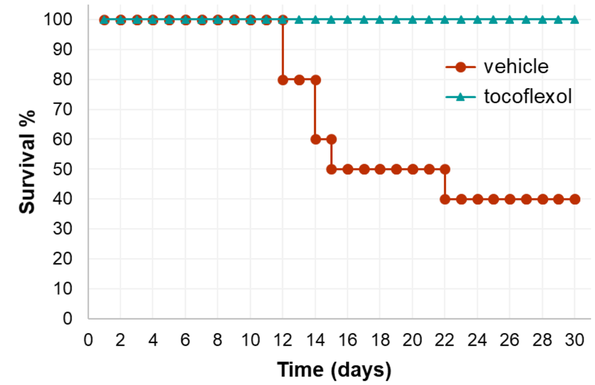

Figure 7. Radioprotection survival study shows survival in mice subcutaneously pre-treated with 300 mg/kg tocoflexol 24 hours before total body irradiation of 9.5 Gy. Mice were monitored for 30 days; n=10.

Conclusions / Summary

- Tocoflexol is a novel tocotrienol analogue designed for improved pharmacokinetics to be a more effective radioprotector/radiomitigator
- Computation modeling and molecular dynamics shows tocoflexol can bind to ATTP similarly to α-tocopherol, whereas tocotrienols cannot
- Pharmacokinetic modeling estimates tocoflexol will have an improved *in vivo* oral bioavailability between α-tocopherol and tocotrienols
- Tocoflexol has high cell uptake comparable with tocotrienols
- Tocoflexol is a potent antioxidant comparable with tocotrienols
- Tocoflexol shows powerful radioprotective activity *in vivo*, with s.c. administration of tocoflexol 24h prior to radiation providing 100% survival to mice exposed to lethal total body irradiation
- Tocoflexol is a promising option to develop into an efficacious radioprotector, and future studies will assess oral administration and its radiomitigation efficacy *in vivo*
- This research was supported in part by grants from the National Center for Research Resources award 1ULI RR029884, the IDeA Networks of Biomedical Research Excellence (INBRE), UAMS start-up funds, and NIH/NIAID grant AI67798