Improving Therapeutic Index of Tylocrebrine through Nanoparticle-Based Reformulation Strategy
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Purpose
The clinical use of many anti-cancer agents has been limited because of severe toxic side effects. The unfavorable physicochemical properties of these drugs require the use of toxic excipients for their formulation. Reformulation strategies that can improve the pharmacokinetics and mitigate non-specific toxicity will allow many such drugs to be reclaimed and decrease the overall anticancer drug development costs. We illustrate here a reformulation approach for tylocrebrine, a phenanthropiperidine alkaloid isolated from T. crebriflora with potent anticancer activity. The clinical use of this molecule was discontinued because of CNS toxicities observed in phase I clinical trials. These side effects likely arise from the CNS penetration of tylocrebrine. We propose that encapsulating tylocrebrine in targeted polymeric nanoparticles will reduce its brain penetration and eliminate the CNS toxicities. The use of polymeric nanoparticles will also obviate the need for toxic excipients such as Cremophor.

Methods
Because many tumor cells overexpress epidermal growth factor receptor (EGFR) on their surface, nanoparticles functionalized with EGFR-targeting peptide was used in our studies. Nanoparticles loaded with tylocrebrine and surface functionalized with carboxy-terminated PEG were first synthesized by solvent evaporation technique. The terminal carboxyl group was then conjugated to the amino terminus of the EGFR targeting peptide using NHS-EDC chemistry. Nanoparticles were characterized for size, zeta potential, drug and peptide loading, and drug release. Cytotoxicity and cell uptake of targeted and non-targeted formulations were determined in vitro.

Results
The reaction conditions were optimized to achieve a balance between particle size (~250 nm), drug loading (4.2%w/w) and peptide conjugation efficiency (38%). Under in vitro conditions (pH 7.4, 37°C), the drug was released over 48 hours. In vitro cytotoxicity studies with A549 lung cancer cells showed that targeted nanoparticles were significantly more potent than non-targeted nanoparticles (IC50 – 24 Vs 64 nM). This increased efficacy was attributable to enhanced tumor cell accumulation of targeted nanoparticles relative to non-targeted nanoparticles.

Conclusion
Encapsulation of tylocrebrine in PLGA nanoparticles is a viable reformulation strategy. This approach does not utilize any toxic excipients. Future studies in a mouse tumor model will help determine the biodistribution, safety, and efficacy of nanoparticle-encapsulated tylocrebrine.