Synthesis and Topical Delivery of Ester and Carbonate Co-Drugs Derived from Tocopherol and Lipoic Acid

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ABSTRACT SUMMARY

Four novel co-drugs designed to protect the skin against oxidative damage have been synthesized in good yields (63-95%). All four co-drugs were formulated and shown to penetrate into the epidermal/dermal (ED) layer of non-viable pig skin. Extraction efficiencies were determined and the co-drugs were shown to be hydrolytically stable in non-viable skin.

INTRODUCTION

We have previously demonstrated that tocopherol (TOC) and lipoic acid (LA) exhibit synergistic antioxidant activity. Topical formulations containing TOC and LA, either alone or in combination, have been previously developed to maintain or restore concentrations of protective antioxidants in the skin. In animal studies, formulations of α-TOC protected skin against erythema induced by ultraviolet radiation (UVR), lipid peroxidation, photoaging, and carcinogenesis. Prodrug esters of TOC analogs have been developed to improve formulation stability and penetration characteristics of TOC. Our new co-drugs, derived from TOC and LA, overcome some of the limitations associated with previously prepared topical formulations of TOC, LA and combinations of these antioxidants. Specifically, our compounds are inherently more stable than formulations of TOC alone because the chemical connection between TOC and LA masks the susceptible phenolic group of TOC, which tends to be chemically unstable, especially in the presence of UVR. The carboxylic acid of LA is also masked in our compounds which eliminates the need for low pH formulations that are prone to causing skin irritation. Finally, these novel compounds allow precise delivery and co-localization of the two antioxidants to the viable epidermis in a single formulation so that the synergistic activity between these compounds can be fully exploited. This work describes the synthesis and characterization of ester and carbonate co-drugs of α- and δ-TOC and LA. Data describing topical delivery and hydrolysis of the formulated co-drugs are presented.

EXPERIMENTAL METHODS

Synthesis of Ester Co-Drugs (TOCE)

A solution of dicyclohexylcarbodiimide (DCC, 1.1mmol) in dichloromethane (DCM, 10 mL) was added in 5 min to a solution of TOC (1mmol), LA (1.25mmol) and DMAP (0.8mmol) in DCM (10 ml) at 0°C under N2. After the addition was completed the cooling bath was removed and the reaction was stirred overnight. The solvent was evaporated and the crude reaction mixture was purified by flash column chromatography on silica gel using hexane:ethyl acetate 100:0-96:4 to afford the pure product as an yellow viscous oil.

Synthesis of Carbonate Co-Drugs (TOCC)

Lipol (LOH) was synthesized according to a procedure described by Saah et. al. Lipol was then coupled to TOC chloroformate (TOCCF) which was synthesized as follows. Pyridine (2 mmols) was slowly added to a solution of triphosgene (1 mmol) in DCM (5mL) at 0°C under N2. A solution of TOC (1mmol) in DCM (5mL) was added to the reaction mixture over 10-15 min. After the addition was completed the cooling bath was removed and the reaction was allowed to stir overnight. The TOCCF in DCM (10mL) was then added to a solution of LOH (1 mmol) in DCM (10 mL) followed by addition of pyridine (8 mmols) at room temperature. The reaction was stirred overnight and the solvent was evaporated. The crude reaction mixture was purified by column chromatography on silica gel using hexane: ethyl acetate 100:0-99:1-98:2-97:3 to afford pure product as a yellow oil. Co-drugs were characterized by APCI-MS, 1H and 13C NMR, and IR spectroscopy.

In vitro Skin Penetration

Skin penetration assays were conducted for 24 h using Franz diffusion cells and previously frozen porcine ear skin as the model tissue. The receptor compartment consisted of phosphate buffer (pH 7.4, 100 mM) with 20% ethanol, and was maintained at 37 ± 0.5°C with magnetic stirring. Formulations of co-drug (1%) containing 20% monoolein with or without 5% of oleic acid were added to the donor compartment (100 mg). After 24h, skin sections were wiped, and tape stripping was performed to separate the stratum corneum (SC) and epidermal and dermal layers (ED). The first tape was discarded, and the others were extracted with acetonitrile with 5% DCM. The remaining ED was cut into small pieces, and homogenized with the extracting solvent, sonicated for 20 min, and filtered. The concentrations of co-drug in SC and ED are indices of cutaneous (or topical) delivery, whereas the concentration in the receptor phase is an index of transdermal delivery.

Extraction Efficiencies and Co-Drug Hydrolysis

Extraction efficiencies of the co-drugs from the skin were determined and the extent of hydrolysis was measured from 1-24 h. Formulations (1%) of each co-drug (n=4) were prepared in isopropyl myristate,
containing 20% penetration enhancer. For each sample, skin (1 cm²) was cut into pieces and placed in an 8 mL screw cap vial. Formulations (1%) of each co-drug (100mg) was added to each vial and kept at 37°C for 24h. Extraction solvent (5ml, ACN:DCM 95:5) was added to the vial and skin was homogenized for 1min. The suspension was vortexed for 30 sec and sonicated for 30 min. Samples were filtered and the filtrate was analyzed by HPLC (287nm, XBridge™ C8 4.6 mm x 150 mm, 3.5μ ACN:MeOH:DCM 60:35:5 and flow rate 0.75ml/min).

RESULTS AND DISCUSSION

The co-drug structures are represented in Figure 1. The ester and carbonates derived from LA or lipol and α- and δ-TOC were prepared in good yield (Table 1). HPLC analysis revealed that each of the co-drugs was clearly resolved from the corresponding TOC (Table 1), demonstrating that hydrolysis of the co-drugs could be easily detected and quantified by HPLC analysis.

![Figure 1: Parent co-drug structure](image)

<table>
<thead>
<tr>
<th></th>
<th>Yield</th>
<th>Rt (min)</th>
<th>% Recovery</th>
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<tbody>
<tr>
<td>α-TOC</td>
<td>--</td>
<td>4.90</td>
<td>89.02</td>
</tr>
<tr>
<td>δ-TOC</td>
<td>--</td>
<td>4.49</td>
<td>92.66</td>
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<tr>
<td>α-TOCE 90%</td>
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<td>δ-TOCE 95%</td>
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<td>α-TOCC 60%</td>
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<td>98.79 ± 4.89</td>
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<tr>
<td>δ-TOCC 56%</td>
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<td></td>
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</tr>
</tbody>
</table>

Table 1: Co-drug Yields, Retention Times and % Recovery (24h) from Skin

Skin penetration of co-drugs in 20% monoolein (MC) and MC with 5% oleic acid (MCOA) after 24h is shown in Figure 2. All co-drugs were detected in the SC and the ED layers, but no co-drug was detected in the receptor phase indicating that no transdermal delivery occurred. No significant differences in penetration were observed among the four co-drugs evaluated however formulations containing oleic acid enhanced penetration of all four co-drugs in the ED layer. This finding is significant as penetration and retention of these co-drugs in the ED is necessary for eventual hydrolysis and release of the synergistic antioxidants.

Co-drug extraction efficiencies from the skin were determined and are shown in Table 1. Excellent recovery of all of the co-drugs from skin was achieved. Hydrolysis was detected for δ-TOCC (2%) and was taken into account when calculating extraction efficiencies. No significant hydrolysis was detected for the other co-drugs.

CONCLUSION

Four co-drugs derived from TOC and LA were synthesized in good yield. All four co-drugs were shown to penetrate into the ED layers of the skin. These co-drugs appear to be stable in the formulations evaluated in a non-enzymatic environment.

REFERENCES

1. Hass, M. A.; Hammad, A. Natural antioxidants act synergistically to inhibit lipid peroxidation. 242nd American Chemical Society Meeting (Division of Toxicology), Denver, CO, August 28-September 1, 2011.

ACKNOWLEDGMENTS

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