Transdermal Dual-Therapy of Metformin and Trans-Resveratrol Nanocochleates with Synergistic Anticancer Effects

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Problem Statement

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality worldwide [1]. Metformin hydrochloride (MET) and trans-resveratrol (T-R) have shown promising anticancer activity against HCC [2,3]. However, the clinical use of MET is limited by its high hydrophilicity and low bioavailability [4], while T-R suffers from poor bioavailability due to low aqueous solubility, chemical instability, and extensive hepatic metabolism [5].

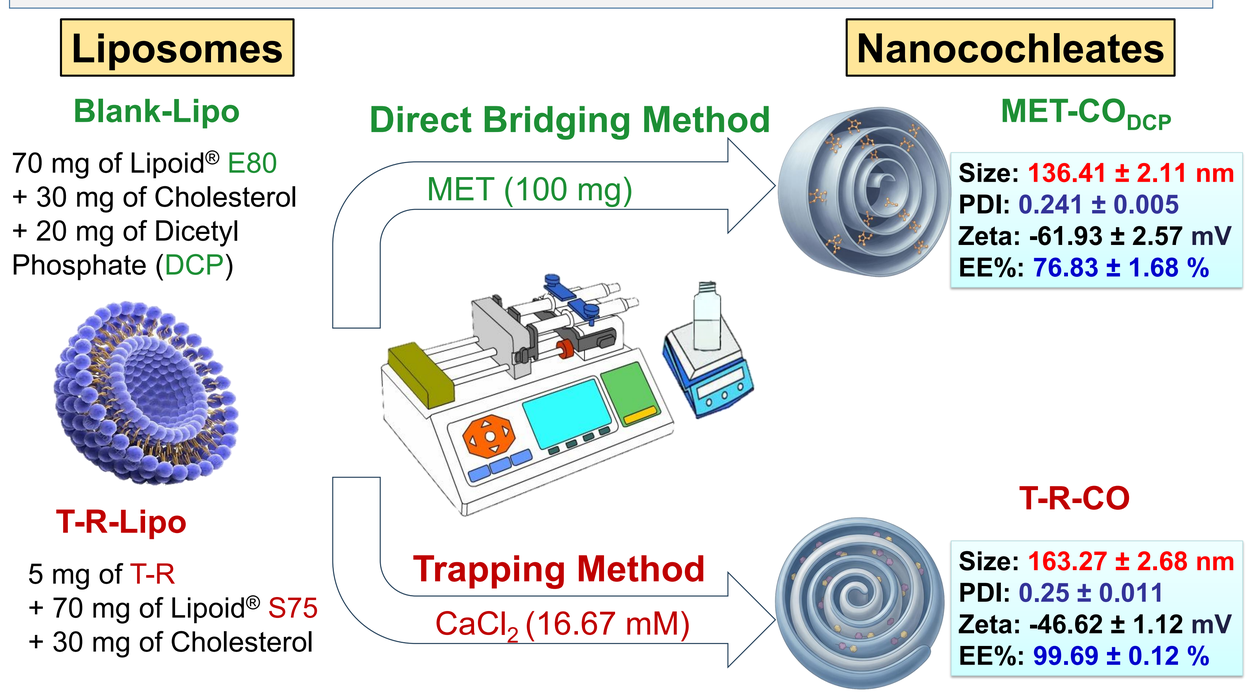
Aim: To develop, for the first time, MET-bridged and T-R–loaded nanocochleates to enhance the transdermal delivery of both drugs, improve drug loading and stability, and potentiate the synergistic anticancer efficacy of dual-drug therapy against HCC.

Methodology

MET-bridged and T-R–loaded nanocochleates were prepared using direct bridging or drug-trapping techniques, respectively. Optimized formulations were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), in-vitro drug release [6,7], UV stability, and incorporation into hydrogel systems. Transdermal permeation was evaluated using an ex-vivo rat skin model. The synergistic anticancer efficacy of dual-drug nanocochleates was assessed in HepG2 cells through cytotoxicity (MTT assay), cellular uptake (CLSM), and gene expression analysis (qRT-PCR).

Results

[1] Nanocochleates formulation



[2] In-vitro characterization

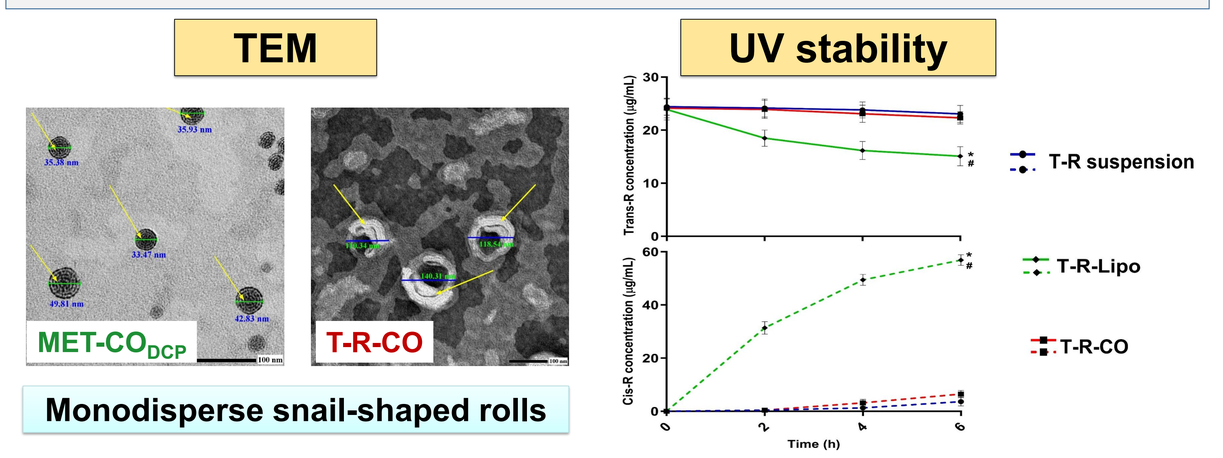


Fig. 1. TEM images of MET-bridged (MET-CO_{DCP}) and T-R-loaded nanocochleates (T-R-CO); scale bars 100 nm. **UV stability** of T-R-loaded nanocarriers (T-R-Lipo and T-R-CO) compared with T-R suspension equivalent to 200 µg T-R (in acetate buffer, pH 5.5) at 25 ± 1℃ for 6 h. Data are expressed as mean ± SD (n = 3). Statistical differences were significant at $p < 0.05$ (* vs. T-R suspension and # vs. T-R-CO).

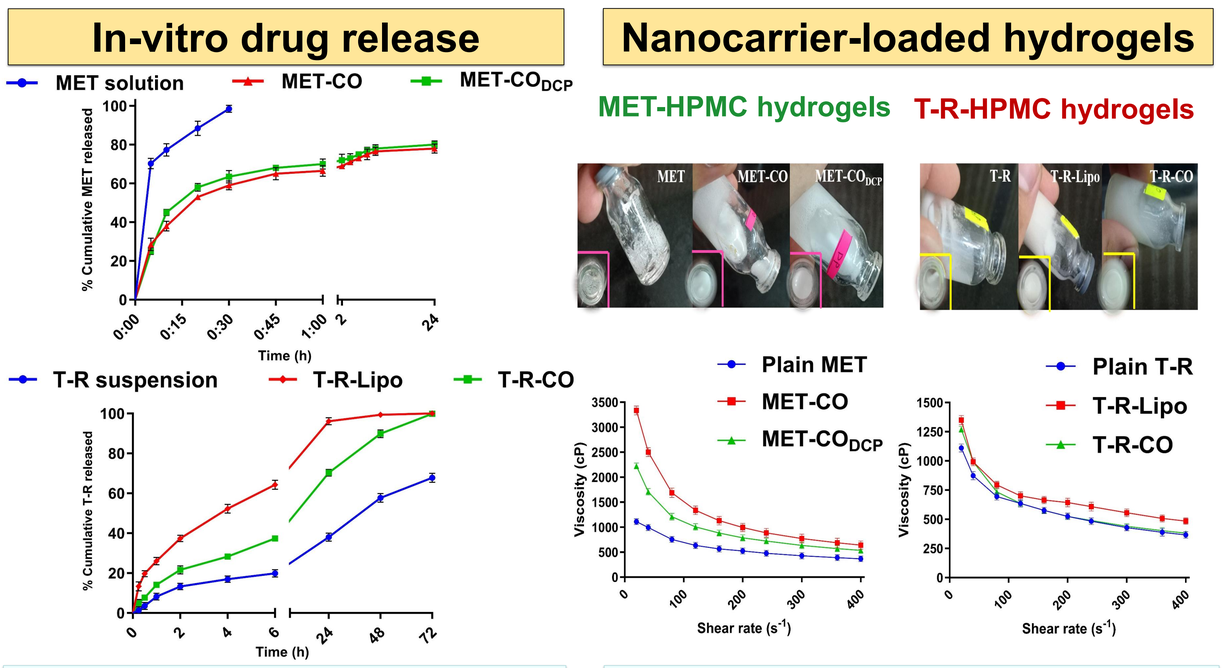


Fig. 2. In-vitro release profiles of MET from its solution (in phosphate buffer, pH 7.4), MET-CO, and MET-CO_{DCP} formulations, alongside that of T-R from its suspension (in acetate buffer, pH 5.5), T-R-Lipo, and T-R-CO formulas (in PBS, pH 6.8) at 100 rpm and 37℃ using dialysis bag method. **Viscosity curves** of 2% HPMC 4000 hydrogels incorporating MET-bridged nanocochleates and T-R-loaded nanocarriers at room temperature. Photographs illustrate the homogeneity of all plain drugs and their respective formula-loaded hydrogels at final MET and T-R concentrations of 20 and 1 mg/mL, respectively, while 0.83 mg/mL in T-R-CO hydrogel. Inset images show top views of the vials. Data are expressed as mean ± SD (n = 3).

[3] Ex-vivo skin permeation and retention

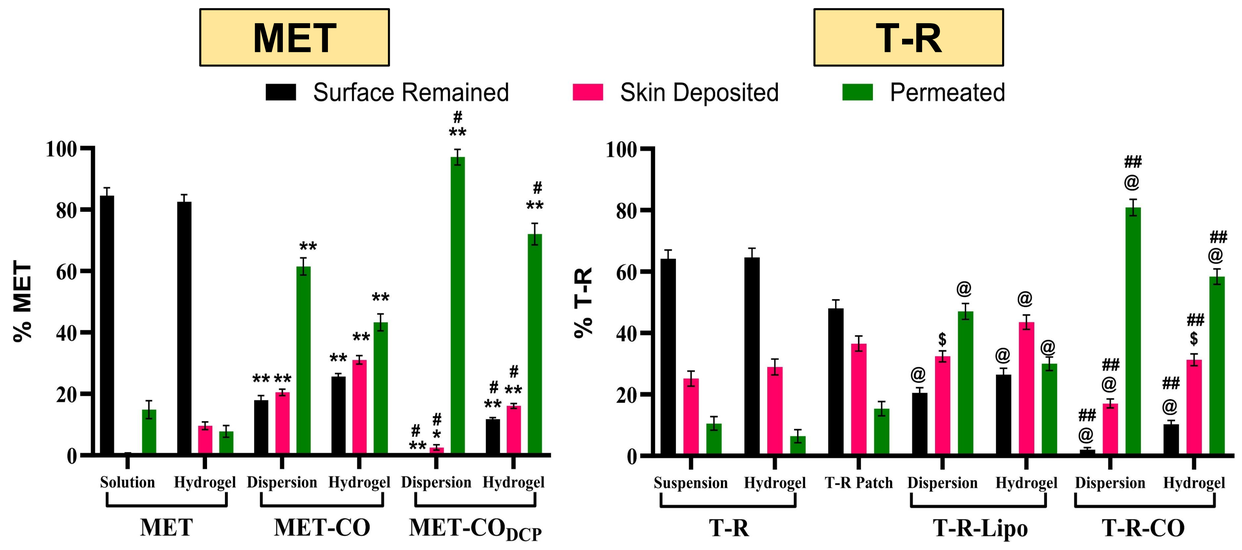


Fig. 3. Ex-vivo skin distribution profiles of MET and T-R formulations after 24 h through rat skin using modified Franz diffusion cell method at pH 6.8 and 32 ± 0.5 °C. Data are expressed as mean ± SD (n = 3). Statistical differences were significant among different dispersions/hydrogels/T-R patch within each section (surface remained, skin deposited and permeated) at $p < 0.05$ (* vs. MET hydrogel, ** vs. MET solution and hydrogel, # vs. MET-CO dispersion and its hydrogel, whereas $ vs. T-R suspension, @ vs. T-R suspension, hydrogel, and T-R patch, ## vs. T-R-Lipo dispersion and its hydrogel).

[4] Anticancer activity

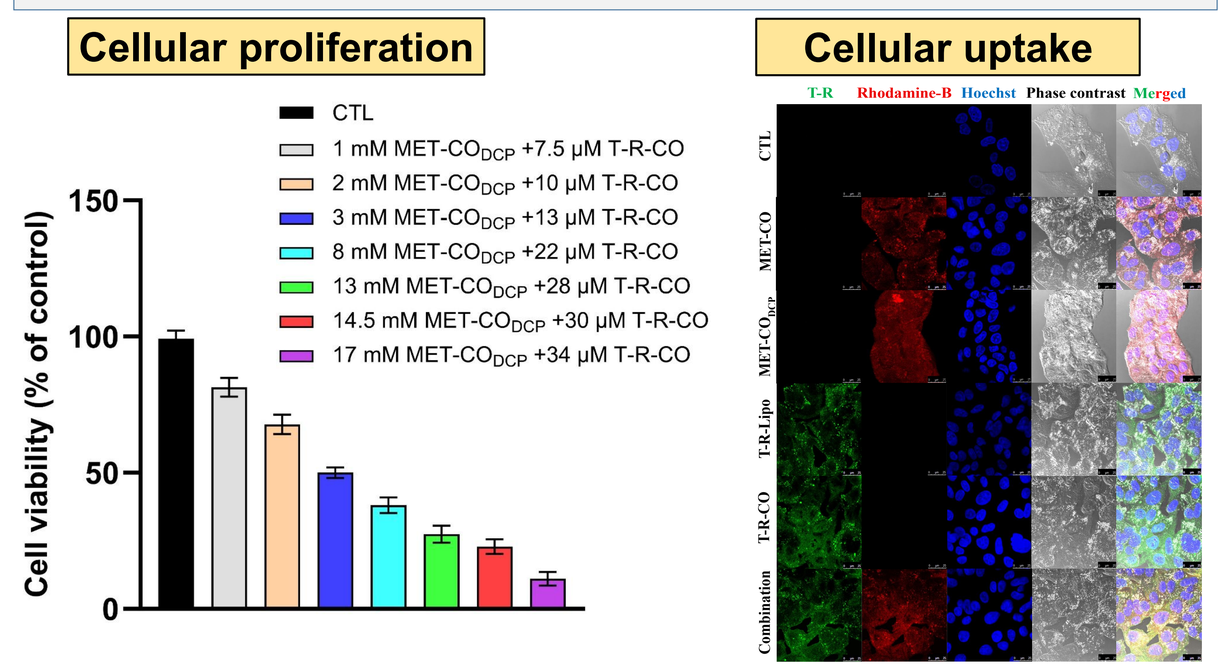


Fig. 4. In-vitro cytotoxicity studies of combined MET-bridged and T-R-loaded nanocochleates on HepG2 cell line. Cells were treated with serial concentrations of combination therapy for 24 h. Data are expressed as mean ± SD (n = 3). **CLSM images showing cellular uptake** of MET-CO, MET-CO_{DCP}, T-R-Lipo, and T-R-CO alongside combined therapy of MET-CO_{DCP} (~3 mM) and T-R-CO (~13 µM) in HepG2 cells after 1 h treatment with their respective 24 h-IC_{50} values. Scale bar 25 µm.

[5] Gene expression

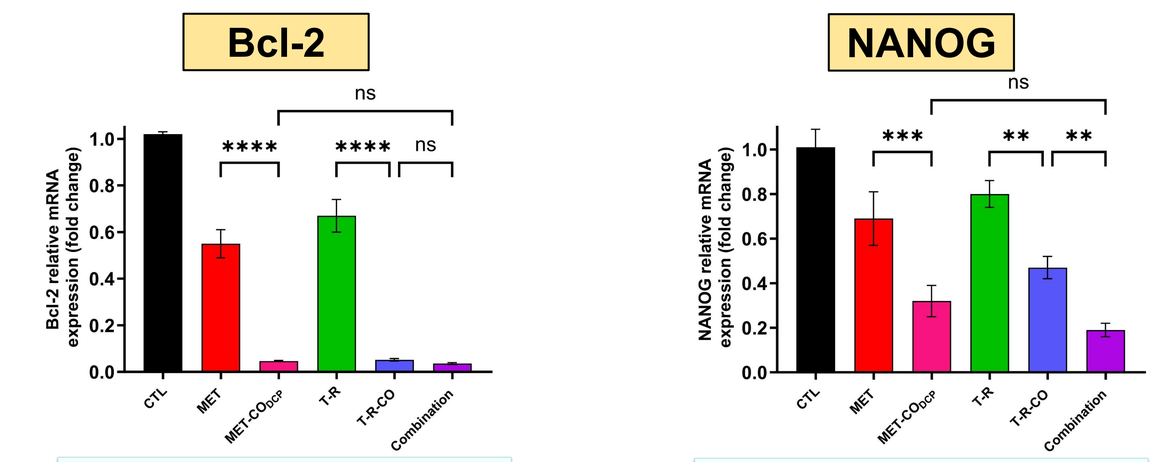


Fig. 5. Suppression of anti-apoptotic (Bcl-2) and cancer stemness (NANOG) biomarker genes in HepG2 cells after 24 h-treatment with 24 h-IC_{50} of each MET (its solution and MET-CO_{DCP}) and 24 h-IC_{50} of T-R (its solution and T-R-CO) and also with half the 24 h-IC_{50} values of both MET and T-R as combination therapy, including MET-CO_{DCP} (~7.4 mM) and T-R-CO (~33 µM). Bcl-2 and NANOG levels were normalized against GAPDH levels. Data are expressed as mean ± SD (n = 3). Statistical differences were significant (** at $p \leq 0.01$, *** at $p \leq 0.001$, and **** at $p \leq 0.0001$).

Conclusions

- Uniform, nanosized cochleate structures with high MET and T-R encapsulation efficiencies were successfully developed.
- The formulations enhanced UV stability of T-R along with controlled release profiles of MET and T-R and were effectively incorporated into HPMC-based hydrogels.
- Ex-vivo studies demonstrated significantly improved transdermal permeation across rat skin.
- Combined MET-bridged and T-R–loaded nanocochleates exhibited enhanced synergistic anticancer activity in HepG2 cells, characterized by efficient cellular uptake and significant downregulation of anti-apoptotic and stemness-related genes Bcl-2 and NANOG.

Acknowledgements: The authors gratefully acknowledge Lipoid AG (Ludwigshafen, Germany) for generously providing Lipoid® S75 and E80.

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