Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro-hyperthermia

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Abstract

Background: Modulated electro-hyperthermia (mEHT) is a form of hyperthermia used in cancer treatment. mEHT has demonstrated the ability to activate host immunity by inducing the release of heat shock proteins, triggering apoptosis, and destroying the integrity of cell membranes to enhance cellular uptake of chemo-drugs in tumor cells. Both curcumin and resveratrol are phytochemicals that function as effective antioxidants, immune activators, and potential inhibitors of tumor development. However, poor bioavailability is a major obstacle for use in clinical cancer treatment.

Methods: This purpose of this study was to investigate whether mEHT can increase anti-cancer efficacy of nanosized curcumin and resveratrol in in vitro and in vivo models. The in vitro study included cell proliferation assay, cell cycle, and apoptosis analysis. Serum concentration was analyzed for the absorption of curcumin and resveratrol in SD rat model. The in vivo CT26/BALB/c animal tumor model was used for validating the safety, tumor growth curve, and immune cell infiltration within tumor tissues after combined mEHT/curcumin/resveratrol treatment.

Results: The results indicate co-treatment of mEHT with nano-curcumin and resveratrol significantly induced cell cycle arrest and apoptosis of CT26 cells. The serum concentrations of curcumin and resveratrol were significantly elevated when mEHT was applied. The combination also inhibited the growth of CT26 colon cancer by inducing apoptosis and HSP70 expression of tumor cells while recruiting CD3+ T-cells and F4/80+ macrophages.

Conclusions: The results of this study have suggested that this natural, non-toxic compound can be an effective anti-tumor strategy for clinical cancer therapy. mEHT can enable cellular uptake of potential anti-tumor materials and create a favorable tumor microenvironment for an immunological chain reaction that improves the success of combined treatments of curcumin and resveratrol.

Keywords: Modulated electro-hyperthermia (mEHT), curcumin, resveratrol, nanosized, apoptosis, tumor microenvironment
Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro- hyperthermia (mEHT)

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Introduction

- mEHT was widely used to promote the synergistic effects in a variety of cancer therapies.
- Both curcumin and resveratrol are phytochemicals that function as effective antioxidants, immune activators, and potential inhibitors of tumor development.
- Poor bioavailability is a major obstacle for the using of curcumin and resveratrol in clinical cancer treatment.
- We have developed a unique platform for nanosized curcumin and resveratrol.
- This purpose of this study was to investigate whether mEHT can increase anti-cancer efficacy of nanosized curcumin and resveratrol.
A unique platform for nanosized curcumin and resveratrol

Nano bead mills

Particle sizes after nanosizing

Before and after nanosizing

Nano formulation of curcumin plus resveratrol enhanced the absorption in serum of rat model

Table 1. Pharmacokinetic parameters derived from rat plasma. *

* AUC: area under the blood concentration vs time curve; $C_{\text{max}}$: maximum concentration; and $T_{\text{max}}$: time to reach $C_{\text{max}}$

| Sample                  | $AUC_{(0-\text{last})}$ (ng*hr/mL) | $C_{\text{max}}$ (ng/mL plasma) | $T_{\text{max}}$ (hr) |
|-------------------------|-----------------------------------|---------------------------------|-----------------------|
| Curcumin suspension     | 46.3 ± 30.7                       | 18.9 ± 20.1                     | 2.5 ± 1.8             |
| Curcumin nanoparticles  | 215 ± 46.4                        | 37.7 ± 21.8                     | 2.17 ± 1.44           |
| Resveratrol suspension  | 1608 ± 284                        | 522 ± 152                       | 2.67 ± 0.58           |
| Resveratrol nanoparticles | 1632 ± 286                    | 782 ± 105                       | 0.83 ± 1.01           |
Nano formulation of curcumin plus resveratrol inhibited the cell viability in CT26

![Graph showing the effect of curcumin and resveratrol on cell viability in CT26.](image)

C: Curcumin
R: Resveratrol

Nano formulation of curcumin plus resveratrol induced cell cycle arrest in CT26

![Graph showing cell cycle distribution in CT26.](image)

Both Cyclin D1 and Cyclin A decreased after CR treatment on CT26 to reveal decreased cell viability was partially due to their damaging cell cycle progression.
Nano formulation of curcumin plus resveratrol with mEHT increased significant apoptosis in CT26

These results showed mEHT combined with curcumin and resveratrol induce cell apoptosis and immunogenic cell death to trigger further immune response.
CT26 tumors were inhibited by nano formulation of curcumin plus resveratrol combined with mEHT treatment

![Graph showing tumor size and treatment groups over 14 days.]

C: Curcumin
R: Resveratrol

These results were in concordance with our *in vitro* findings and indicated that curcumin and resveratrol oral administration combined mEHT treatment could significantly suppress tumor growth.
Increased infiltration of macrophages and T-lymphocytes were observed in tumors treated by CR and mEHT combination

This indicates that in addition to reduced tumor cell viability, combined treatment of CR and mEHT could also trigger host immunity by recruiting T-cells and macrophages.

Increased of Hsp70 expression was observed in tumors treated by CR and mEHT combination

This results support our hypothesis that potential immune activation was induced by CR treatment and mEHT for CT26 tumor eradication.
Conclusions

- This study indicates that nano-formulated curcumin plus resveratrol compound shows enhanced bioavailability when combined with mEHT, synergistically increasing HSP-release and immune response, leading to enhanced anti-tumor efficacy in CT26 tumors.
- Further clinical studies are needed to confirm the safety and effectiveness of nano-formulated curcumin and resveratrol when combined with mEHT.

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RESEARCH ARTICLE

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