Cytotoxic Activity of the New Molecular Complex DHMEQ and Ammonium Glycyrrhizinate

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Abstract: *Objective*: This study aimed to investigate the cytotoxic activity of a new molecular complex consisting of DHMEQ and ammonium glycyrrhizinate, as well as its effect on the transcription factor NF-κB. Cytotoxicity was assessed using the PrestoBlue® viability assay in HEK293, A-549, and MCF-7 cell lines. NF-κB inhibition was evaluated via a luciferase reporter assay in HEK293 cells. The complex was prepared at a 1:4 molar ratio (DHMEQ:ammonium glycyrrhizinate), and its structure was confirmed using spectroscopic methods and electron microscopy. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test.

Results: DHMEQ demonstrated high cytotoxic activity (IC_{50} = 13.82 ± 3.71 μM in HEK293 cells). The DHMEQ/ammonium glycyrrhizinate complex maintained comparable activity (IC_{50} = 10.39 ± 1.84 μM for HEK293) but showed reduced efficacy against A-549 and MCF-7 tumor cells. DHMEQ strongly inhibited NF-κB activity (IC_{50} = 0.83 ± 0.51 μM), while the complex required significantly higher concentrations (IC_{50} = 21.79 ± 6.24 μM) to achieve a similar inhibitory effect.

Conclusion: The DHMEQ–ammonium glycyrrhizinate complex preserved the main biological properties of DHMEQ while improving its solubility and stability. This approach shows potential for developing DHMEQ-based drug formulations targeting NF-κB, but further optimization and *in vivo* validation are required before clinical application.

Keywords: Cytotoxicity, NF-κB inhibition, DHMEQ, ammonium glycyrrhizinate, molecular complex, transcription factor, *in vitro*, drug development.

INTRODUCTION

The transcription factor NF-κB plays a central role in regulating inflammation, cell proliferation, and apoptosis, making it an attractive target for anticancer and anti-inflammatory drug development, particularly for overcoming treatment resistance [1]. Hyperactivation of NF-κB is associated with tumor progression, immune evasion, treatment resistance, and poor clinical outcomes [2-5].

DHMEQ (dehydroxyethylquinomycin) is a selective NF-κB inhibitor known for suppressing its activity without significantly affecting other signaling pathways [6]. Preclinical studies have demonstrated its potent antitumor and anti-inflammatory effects [7]. However, DHMEQ's clinical application is limited by poor solubility, pharmacokinetic instability, and toxicity at higher concentrations [8].

Recent strategies focus on developing new formulations (e.g., nanocarriers) to enhance bioavailability, stability, and therapeutic efficacy of NF-κB inhibitors [9]. One promising approach involves creating molecular complexes by combining DHMEQ with additional components that improve stability and delivery [10, 11].

In this study, we evaluated a DHMEQ--ammonium glycyrrhizinate complex. HEK293 cells (human embryonic kidney; non-tumor), A-549 cells (lung adenocarcinoma), and MCF-7 cells (breast adenocarcinoma) were selected for comprehensive evaluation. HEK293 cells were chosen to assess baseline cytotoxicity in non-malignant models, whereas A-549 and MCF-7 represent distinct tumor types with varying NF-κB activation levels, providing insight into the potential therapeutic applicability of the complex.

OBJECTIVE OF THE STUDY

To evaluate the cytotoxic activity of the DHMEQ–ammonium glycyrrhizinate molecular complex on different human cell lines *in vitro* and compare its efficacy with unmodified DHMEQ, focusing on its ability to inhibit NF-κB activity.

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MATERIALS AND METHODS

Cell Lines

Three human cell lines were obtained from the Russian Collection of Cell Cultures (Institute of Cytology, RAS, St. Petersburg):

- HEK293: human embryonic kidney cells, widely used due to their high transfectability and reproducibility.
- A-549: human lung adenocarcinoma cells, a model for studying cancer cell biology and drug screening.
- MCF-7: human breast adenocarcinoma cells, commonly used to assess drug effects in hormone-dependent tumors.

Preparation of the Molecular Complex

The DHMEQ/ammonium glycyrrhizinate complex was prepared by mixing the components in 70% ethanol at a 1:4 molar ratio. The mixture was heated to boiling, followed by solvent evaporation using a rotary evaporator. The resulting residue was vacuum-dried to obtain a beige cylindrical powder. Particle size distribution was assessed via dynamic light scattering (DLS). The formation of the complex was confirmed using UV-visible spectroscopy and electron microscopy, demonstrating stable molecular association and defined particle morphology.

Cell Culture

Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 50 μg/mL gentamicin at 37°C in a 5% CO_2 incubator. Seeding densities in 96-well plates were 10×10^3 cells/well for A-549 and MCF-7, and 25×10^3 cells/well for HEK293.

Treatments and Viability Assay

Upon 24 h incubation (monolayer formation), cells were treated with DHMEQ, the DHMEQ/AG complex, or ammonium glycyrrhizinate across 1, 10, and 100 μM (DMSO ≤ 0.1%). After 48 h, cell viability was quantified using PrestoBlue® (Invitrogen, USA) according to the manufacturer's protocol. Fluorescence signals were measured on a spectrofluorometer and normalized to vehicle controls to calculate metabolic activity and IC_{50} values (Table **1**).

Cytotoxicity Assessment

Cell viability was assessed 48 hours post-treatment using the PrestoBlue® viability assay (Invitrogen, USA). Fluorescence intensity was measured using a spectrofluorometer and normalized to control wells. IC_{50} values were calculated based on cell metabolic activity.

NF-κB Inhibition Assay

In HEK293 cells, NF-κB DNA-binding activity was quantified using a luciferase reporter system across increasing concentrations of DHMEQ and the complex, enabling determination of IC_{50} for NF-κB inhibition (Table **2**, Figure **1**).

Statistical Analysis

Data are expressed as mean ± SD. Statistical comparisons used one-way ANOVA with Dunnett's post hoc test; $p < 0.05$ was considered significant. Each experiment was performed in two independent series with the minimum number of technical replicates ($n \geq 3$) required to ensure statistical reliability, as specified in the Russian protocol.

RESULTS

Cytotoxicity of DHMEQ and the Complex

Across the three lines, DHMEQ exhibited pronounced cytotoxicity, with the highest sensitivity in HEK293 cells (IC_{50} = 13.82 ± 3.71 μM). Tumor lines were less sensitive: A-549 IC_{50} = 24.89 ± 2.33 μM (p = 0.0013 vs HEK293) and MCF-7 IC_{50} = 79.39 ± 2.98 μM (p = 0.00001 vs HEK293). The DHMEQ/ammonium glycyrrhizinate (AG) complex preserved this pattern and overall potency: HEK293 IC_{50} = 10.39 ± 1.84 μM; A-549 IC_{50} = 22.28 ± 4.11 μM (p = 0.002 vs HEK293); MCF-7 IC_{50} = 82.56 ± 2.50 μM (p = 0.00001 vs HEK293). Ammonium glycyrrhizinate alone showed no cytotoxicity up to 100 μM. These data are summarized in Table **1** and indicate that the complex retains the pharmacological activity of DHMEQ, especially in HEK293 and A-549, while MCF-7 cells remain comparatively less responsive, consistent with line-specific survival signaling.

NF-κB Inhibition

In HEK293 cells, DHMEQ strongly inhibited NF-κB activity with IC_{50} = 0.83 ± 0.51 μM. The DHMEQ/AG complex also reduced NF-κB activity but required markedly higher concentrations to achieve a

Table 1: Cytotoxic Effects of DHMEQ and the DHMEQ/Ammonium Glycyrrhizinate Complex *In vitro*

No.	Compound	Solubility (100% DMSO)	IC50 (μM)		
			Hek293	A-549	MCF-7
1	DHMEQ	+	13.82 ± 3.71	24.89 ± 2.33 (p=0.0013)	79.39 ± 2.98 (p=0.00001)
2	DHMEQ/ammonium glycyrrhizinate (1:4)	+	10.39 ± 1.84	22.28 ± 4.11 (p=0.002)	82.56 ± 2.50 (p=0.00001)
3	Ammonium glycyrrhizinate	+	>100	>100	>100

Table 2: NF-κB DNA-Binding Inhibition in HEK293 Cells

No.	Compound	Concentration Range (μM)	IC50 (μM)
1	DHMEQ	0.1, 1, 10	0.83 ± 0.51
2	DHMEQ/ammonium glycyrrhizinate (1:4)	0.5, 5, 50	21.79 ± 6.24* (p=0.001)
3	Ammonium glycyrrhizinate	1, 10, 100	> 100

comparable effect (IC_{50} = 21.79 ± 6.24 μM, $p < 0.05$ vs DHMEQ). Ammonium glycyrrhizinate alone had no measurable inhibition up to 100 μM. These data are presented in Table **2** and illustrated in Figure **1**.

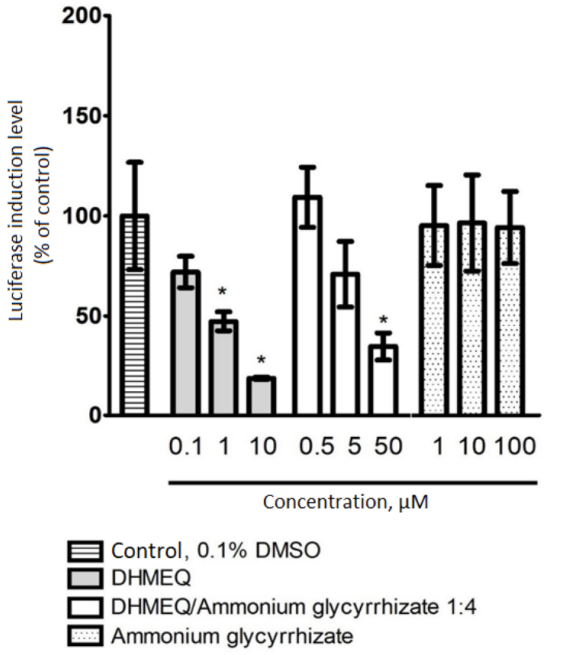

Figure 1: Evaluation of NF-κB DNA-binding inhibition by DHMEQ and the DHMEQ/ammonium glycyrrhizinate complex in HEK293 cells (data not shown).

DISCUSSION

Comparative Cytotoxicity and Cell-Line Sensitivity. The DHMEQ/AG complex retained cytotoxic efficacy comparable to DHMEQ in HEK293 and A-549 cells and only slightly underperformed in MCF-7, mirroring DHMEQ's lower potency in this line. The heightened sensitivity of HEK293 (non-tumor) compared with tumor lines may reflect differences in baseline NF-κB activity, stress responses, or metabolic context. In MCF-7, relative resistance could be linked to pro-survival signaling, distinct regulatory networks, and epigenetic modifications [12] that attenuate DHMEQ-mediated cytotoxicity, a phenomenon often observed in breast cancer models with constitutive NF-κB activity [13], warranting further mechanistic analysis.

Role of Ammonium Glycyrrhizinate in the Complex. Ammonium glycyrrhizinate (AG) lacked intrinsic cytotoxicity up to 100 μM, supporting its auxiliary role, which is consistent with known properties of glycyrrhizic acid and its derivatives [14]. Its key contribution likely lies in enhanced solubility and stabilization of DHMEQ in aqueous environments, improving dispersion – a common approach for bioavailability enhancement of poorly soluble compounds [15] – and potentially mitigating aggregation or rapid degradation. These formulation-relevant attributes are valuable for drug development, where improved physicochemical properties often translate into better bioavailability and more predictable exposure.

NF-κB Inhibition: Preserved Mechanism with Lower Apparent Potency

DHMEQ's potent NF-κB inhibition ($IC_{50} \approx 0.83$ μM) was preserved qualitatively in the complex, yet higher doses were needed ($IC_{50} \approx 21.8$ μM). Several non-exclusive explanations are plausible:

1. Partial sequestration of DHMEQ within the complex reduces immediate availability to interact with NF-κB components;

2. Slower release kinetics from the complex delay or limit target engagement within the assay window;

3. Microenvironmental effects (e.g., local partitioning, protein binding) alter effective intracellular concentrations. Despite this shift in apparent potency, the mechanistic action remains present, supporting the complex as a platform for further optimization rather than as an endpoint formulation.

Despite this shift in apparent potency, the mechanistic action remains present, supporting the complex as a platform for further optimization, potentially involving advanced delivery systems [16], rather than as an endpoint formulation.

Practical Significance and Development Prospects

From a formulation standpoint, preserving DHMEQ's cytotoxic effects while improving solubility/stability is a meaningful advance. The complex can be considered a prototype delivery form that may reduce required excipients, facilitate parenteral or localized administration, and potentially improve safety margins by enabling more controlled exposure. To enhance NF-κB inhibitory potency, next steps could include:

- Tuning the molar ratio and interaction strength within the complex;
- Chemical modification to encourage controlled release;
- Targeted delivery (e.g., ligand-decorated carriers, nanoparticles) to increase tumor exposure while minimizing systemic burden [16];
- Combination strategies with adjuvants that sensitize tumor cells to NF-κB blockade [17] or with immunotherapeutic agents [18].

Collectively, these directions align with the goal of translating DHMEQ's biological promise into clinically viable regimens.

STUDY LIMITATIONS

This work is limited to *in vitro* models. While experiments were performed in two independent series with the minimum number of replicates sufficient for statistical confidence, broader replication would strengthen inference. The absence of *in vivo* data precludes conclusions on pharmacokinetics, biodistribution, and safety. Future studies should include animal models to confirm antitumor activity, characterize exposure–response relationships, and assess tolerability for the DHMEQ/AG complex, as recommended in recent methodological reviews [19].

CONCLUSION

This study evaluated the biological properties of a newly developed molecular complex composed of DHMEQ and ammonium glycyrrhizinate. Using three human cell lines — non-tumor (HEK293) and tumor-derived (A-549 and MCF-7) — we found that the complex largely retains the cytotoxic activity of DHMEQ alone. Comparable effects were observed in HEK293 and A-549 cells, while a slightly reduced response in MCF-7 may reflect cell line–specific resistance mechanisms or differences in NF-κB activation.

An additional finding of this study is that the inclusion of ammonium glycyrrhizinate improved the solubility and apparent stability of DHMEQ, which is consistent with the known properties of glycyrrhizic acid derivatives [14] and strategies for enhancing bioavailability [15]. Although ammonium glycyrrhizinate itself lacked cytotoxicity, its role in stabilizing the compound and facilitating its dispersion in aqueous environments could be relevant for improving DHMEQ formulation properties.

However, our results also show that the NF-κB inhibitory activity of the complex is significantly lower compared to DHMEQ. Whereas DHMEQ achieved strong suppression of NF-κB DNA-binding activity at submicromolar concentrations (IC_{50} ≈ 0.83 μM), the complex required substantially higher concentrations (IC_{50} ≈ 21.79 μM) to produce a comparable effect. This suggests that the biological availability of DHMEQ within the complex is reduced, potentially due to delayed release or partial sequestration.

These findings indicate that while the DHMEQ/ammonium glycyrrhizinate complex demonstrates acceptable cytotoxicity and improved physicochemical properties, it is not yet optimized for efficient NF-κB inhibition. Further research is required to refine the composition, investigate release dynamics, and evaluate its mechanism of action more thoroughly in tumor-specific contexts, including *in vivo* models [19].

In summary, the DHMEQ/ammonium glycyrrhizinate complex represents a potentially useful formulation approach that warrants further optimization and validation. The preservation of cytotoxic properties combined with improved solubility makes it a reasonable starting point for continued development, but the reduced NF-κB inhibition highlights the need for cautious interpretation and additional preclinical research.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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