Cyclosporin A, a non-genotoxic carcinogen – its possible mechanisms of action

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OVERVIEW

Non-genotoxic carcinogens induce cancer without directly affecting DNA. Instead of causing mutations, they disrupt cellular processes like cell cycle regulation, proliferation, epigenetics, inflammation, or oxidative stress, leading to cancer.

AIM

To investigate the mechanism of action of **Cyclosporin A**, IARC group 1 (non-genotoxic) carcinogen and a non-carcinogenic **Ampicillin trihydrate.**

CAN EXISITING MODELS ADEQUATELY IDENTIFY NON-GENOTOXIC CARCINOGENS?

➡ Carcinogenicity studies are traditionally focused on detecting DNA damage, posing a risk of non-genotoxic carcinogens being undetected and consequentyl unregulated.

➡ There is an urgent need to establish new reliable *in vitro* methodologies to detect NgtxC and discover their MoA.

CONCLUSIONS

➡ **Non-genotoxic carcinogen Cyclosporin A did not influence cell viability at tested conditions.**

➡ **Cyclosporin A induced moderate, however insignificant increase in G0/G1 cell number and yH2AX.**

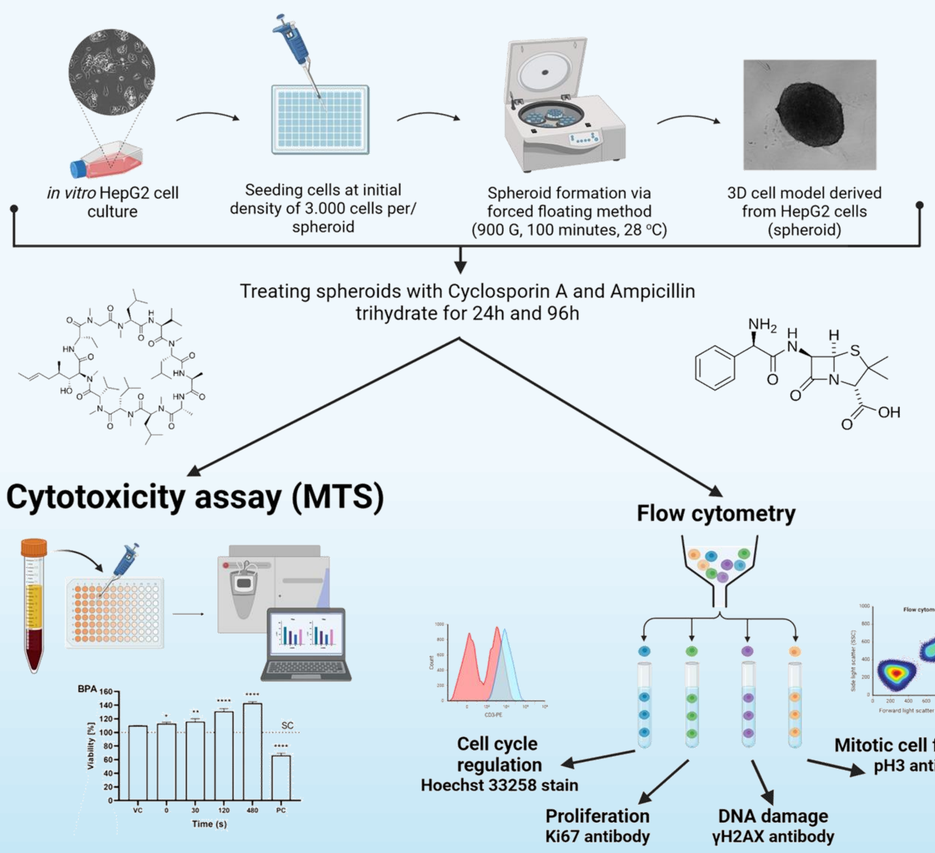
➡ **Cyclosporin A decreased cell proliferation dose and time dependently.**

➡ **Cyclosporin A did not impact mitotic cell formation.**

FUTURE STEPS

Transcriptomic analysis of genes involved in multiple cellular processess (oxidative stress, xenobiotic metabolism, apoptotsis,...), **aiming to identifiy molecular pathways leading to non-genotoxic induced oncogenic changes.**

Workflow



Results

Cyclosporin A

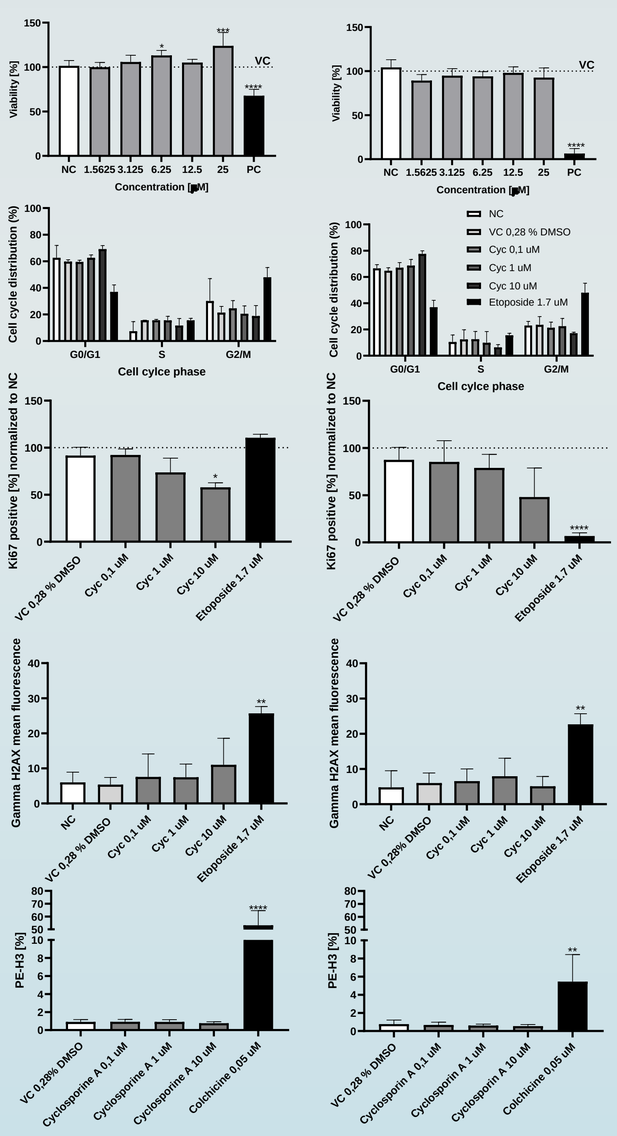


Figure 1: Viability of HepG2 cells in spheroids (MTS assay) after 24h and 96h exposure to Cyclosporin A and Ampcillin trihydrat. PC—positive control (15% DMSO). * significantly different from solvent control, * $p < 0.05$; *** $p < 0.001$; **** $p > 0.0001$ (one-way ANOVA; Dunnett's multiple comparison test).

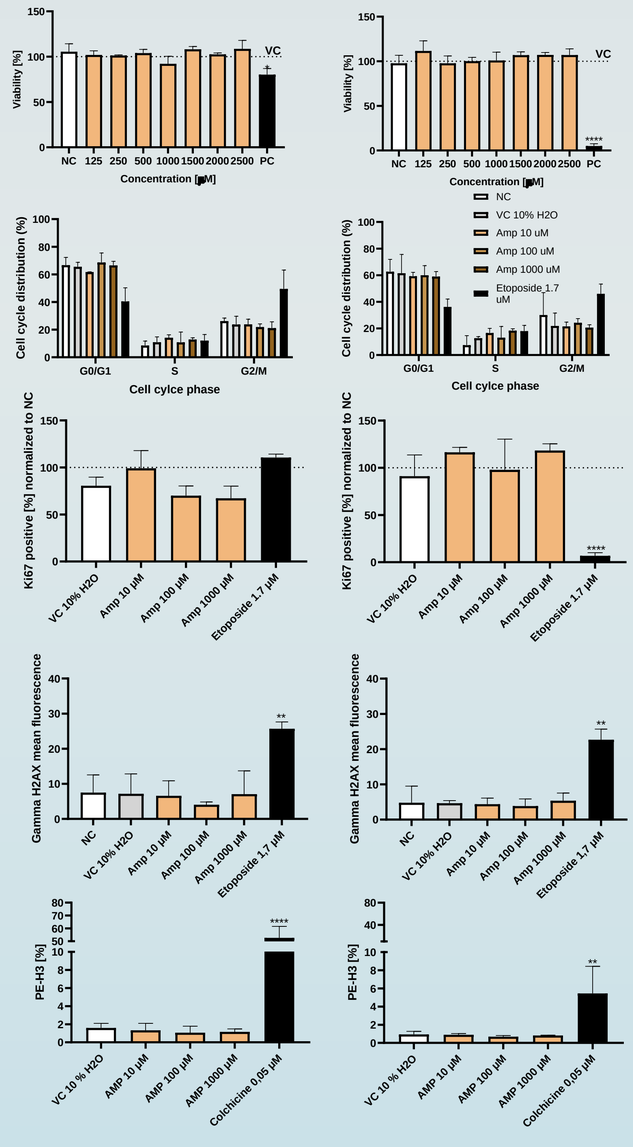
Figure 2: The % of HepG2 cells from spheroids across cell cycle phases after exposure to Cyclosporin A and Ampicillin trihydrate after 24h and 96h. Etoposide (1.7 μM) was a positive control (PC). The % of cell cycle distribution is presented as mean (N=3).

Figure 3: Percentage of Ki67 positive cells normalized to negative control after exposure to Cyclosporin A and Ampicillin trihydrate for 24h 96h. * significantly different from solvent control, * $p < 0.05$; ** $p < 0.01$; **** $p > 0.0001$ (one-way ANOVA; Dunnett's multiple comparison test).

Figure 4: Mean fluorescence corresponding to anti-γH2AX labeled sites after exposure to Cyclosporin A and Ampicillin trihydrate for 24h and 96h. * significantly different from solvent control, * $p < 0.05$; ** $p < 0.01$; (one-way ANOVA; Dunnett's multiple comparison test).

Figure 5: Percentage of pH3 positive cells after exposure to Cyclosporin A and Ampicillin trihydrate for 24h and 96h. * significantly different from solvent control, **** $p > 0.0001$ (one-way ANOVA; Dunnett's multiple comparison test).

Ampicillin trihydrate



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