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Dihydroartemisinin induces endoplasmic reticulum stress-mediated apoptosis in HepG2 human hepatoma cells

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ABSTRACT

Aims and background. Previous studies showed that dihydroartemisinin (DHA) possessed antitumor activity in many human tumor cells through the induction of apoptosis. The aim of this study was to investigate the effects of DHA on apoptosis in the human hepatocellular carcinoma cell line HepG2 and the possible molecular mechanisms involved.

Methods. The inhibitory effect of DHA on HepG2 cells was measured by MTT assay. The percentage of apoptotic cells was detected by flow cytometry with double staining of fluorescein isothiocyanate-annexin V/propidium iodide. The intracellular production of reactive oxygen species (ROS) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were detected by fluorescence spectrophotometry. Protein expression of GADD153, Bcl-2 and Bax in HepG2 cells was examined by Western blot and immunocytochemistry.

Results. DHA significantly inhibited proliferation of HepG2 cells in a dose- and time-dependent manner. The apoptosis rates in HepG2 cells treated with 0, 50, 100 and 200 mol/L DHA for 24 hours were 2.53 ± 0.88%, 24.85 ± 3.63%, 35.27 ± 5.92% and 48.53 ± 7.76%, respectively. Compared with the control group, DHA significantly increased ROS generation and $[Ca^{2+}]_i$ level (P <0.05), with the generation of ROS preceding the increase in $[Ca^{2+}]_i$. An increase in GADD153 and Bax expression and a decrease in Bcl-2 were observed in DHA-treated cells. Pretreatment with the antioxidant N-acetylcysteine could attenuate the effects of DHA in the experiments.

Conclusion. DHA could inhibit proliferation and induce apoptosis in HepG2 cell lines through increasing the intracellular production of ROS and $[Ca^{2+}]_i$. Endoplasmic reticulum stress-induced apoptosis may contribute to this effect by regulating the expression of GADD153, proapoptotic Bax, and antiapoptotic Bcl-2.

Key words: dihydroartemisinin, apoptosis, endoplasmic reticulum stress, reactive oxygen species, calcium, GADD153, Bcl-2, Bax.

Abbreviations

A: absorbance
ATF6: activation of transcription factor 6
$[Ca^{2+}]_i$: intracellular Ca^{2+} concentration,
DCFH: 2′,7′-dichlorofluorescein diacetate
DHA: dihydroartemisinin
DMSO: dimethyl sulfoxide
ER: endoplasmic reticulum
FACS: fluorescence-activated cell sorter
FCM: flow cytometry
FITC: fluorescein isothiocyanate
GADD153: growth-arrest-and-DNA-damage-inducible gene 153
HCC: hepatocellular carcinoma
IR: cell proliferation inhibition rate
IRE1: inositol-requiring transmembrane kinase and endonuclease 1
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAC: N-acetylcysteine
PERK: protein kinase-like ER kinase
PI: propidium iodide
ROS: reactive oxygen species
SERCA: sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
UPR: unfolded protein response

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